FACTORS INFLUENCING ODOR SENSITIVITY IN THE DOG

David G. Moulton

Pennsylvania University

Prepared for:

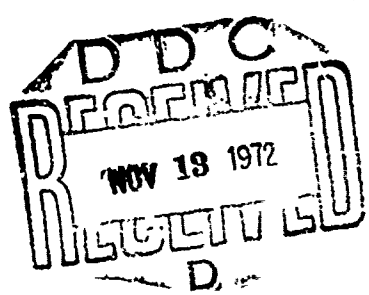
Air Force Office of Scientific Research

October 1972

FACTORS INFLUENCING ODOR SENSITIVITY IN THE DOG

Final Report, October, 1972

Prepared for the Air Force Office of Scientific Research
Contract F 44 620-70-C-0110



D. G. Moulton
Principal Investigator
Monell Chemical Senses Center
University of Pennsylvania

UNCLASSIFIED
Security Classification

DOCUMENT CONTROL DATA - R & D
(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1. ORIGINATING ACTIVITY (Corporate author)
University of Pennsylvania
Monell Chemical Senses Center
Philadelphia, Pennsylvania 19104

2a. REPORT SECURITY CLASSIFICATION
UNCLASSIFIED

2b. GROUP

3. REPORT TITLE
FACTORS INFLUENCING ODOR SENSITIVITY IN THE DOG

4. DESCRIPTIVE NOTES (Type of report and inclusive dates)
Scientific Final

5. AUTHOR(S) (First name, middle initial, last name)
David G. Moulton

6. REPORT DATE	7a. TOTAL NO. OF PAGES	7b. NO. OF REFS
October 1972	~~79~~ 80	38

8a. CONTRACT OR GRANT NO.	9a. ORIGINATOR'S REPORT NUMBER(S)
F44620-70-C-0110	
b. PROJECT NO. 9777	
c. 61102F	9b. OTHER REPORT NO(S) (Any other numbers that may be assigned this report)
d. 681312	AFOSR - TR - 72 - 2050

10. DISTRIBUTION STATEMENT
Approved for public release; distribution unlimited.

11. SUPPLEMENTARY NOTES	12. SPONSORING MILITARY ACTIVITY
TECH,OTHER	Air Force Office of Scientific Research (NL) 1400 Wilson Boulevard Arlington, Virginia 22209

13. ABSTRACT

The development of methods for investigating odor preference in dogs and the relation of preference to performance in learning an odor detection task is described. Four German shepherds were given access to two water bowls or troughs, one associated with a test odorant, the other with a "blank". Consistent preferences were observed when additional criteria of response included the number of entries made into each station and the amount of time spent at each station. The apparatus and technique for training dogs to avoid odors provides an effective method for grouping dogs according to their ability to learn an odor detection task. This series of studies suggests that in certain cases it may be possible to predict performance on the task detection task from simple measures of preference behavior. A programmed apparatus for obtaining accurate quantitative information on the dog's sensitivity to odors is also described. It consists of a 3-choice (odor/air/air) automated discrimination box supplied by a 6 stage air-dilution olfactometer contained in a controlled environment chamber. Concentration may be an important variable in screening dogs for performance in an odor-detection task. Preliminary evidence suggests that the sensiti ity of the dog for alpha-ionone is at least 1,000 - 10,000 times greater than that of untrained human subjects tested in the same apparatus.

Ia

DD FORM 1 NOV 65 1473

UNCLASSIFIED
Security Classification

14. KEY WORDS	LINK A		LINK B		LINK C	
	ROLE	WT	ROLE	WT	ROLE	WT
Dogs						
Olfactory sensitivity						
Odor detection						
Odor preference						
Alpha-ionone						
Hormones						
Estrogen						
Testosterone						
Rats						
Olfactometer						

16

TABLE OF CONTENTS

LIST OF ILLUSTRATIONS

SUMMARY

This report is in three parts. Part I describes the development of methods for investigating odor preference in dogs and the relation of preference to performance in learning an odor detection task. In a series of experiments four female and one male German shepherds were given access to two water bowls or troughs, one associated with a test odorant, the other with a "blank." No stable preferences (not associated with place alone) were seen when the only criterion of response was the amount of water consumed from each station under the following conditions: (1) Dogs were placed on a water deprivation schedule and given access to the test apparatus during test sessions only. (2) Dogs were given continuous access to the test apparatus. Consistent preferences were observed, however, when two additional criteria of response were introduced into the last situation: the number of entries made into each station (blank or odor) and the amount or time spent at each station. Thus one dog preferred blank to benzyl benzoate, butyric acid (10^{-3}) to blank, and valeric acid (10^{-3}) to blank, while all remaining dogs showed the opposite preferences (with the exception of one dog that also preferred butyric acid to blank). The same dogs were then placed on a water deprivation schedule and trained first to avoid valeric acid (10^{-2}) and then to avoid alpha-ionone. The dog showing the reverse preference to the remainder in the previous experiment was also the dog showing the poorest performance on each of these learning tasks. The dogs did not generalize readily from valeric acid to alpha ionone. (This criterion could be used in defining

the degree of similarity of different odors for the dog.) The apparatus and technique for training dogs to avoid odors provides an effective method for grouping dogs according to their ability to learn an odor detection task. This series of studies suggests that in certain cases it may be possible to predict performance on the task detection task from simple measures of preference behavior.

In Part II a programmed apparatus for obtaining accurate quantitative information on the dog's sensitivity to odors is described. It consists of a 3-choice (odor/air/air) automated discrimination box supplied by a 6 stage air-dilution olfactometer contained in a controlled environment chamber. Four dogs were trained to detect alpha-ionone on this apparatus. While the relative performance of the dogs fell in a relatively narrow range for each of the concentration tested between 10^{-3} and 10^{-6} of saturation at 23°, a wide separation of performance occurred at 10^{-7} suggesting that concentration may be an important variable in screening dogs for performance in an odor-detection task. Preliminary evidence suggests that the sensitivity of the dog for alpha-ionone is at least 1,000 - 10,000 X greater than that of untrained human subjects tested in the same apparatus. The weber fraction for flow rate discrimination in these dogs lay between .12 and 0.08.

Part III concerns the alterations in the detectability of odor that occurs as a function of hormonal levels in the rat. Rats were trained to detect cyclopentanone (10^{-3} of saturation) in a two-choice (odor-air) apparatus similar to that described in Part II. Female rats show marked fluctuations in their performance which correlate with the rat sexual

cycle the peak occurring on the day of ovulation. Similar fluctuations occur in responses to eugenol, alpha-ionone and Exaltolide. Males do not show these variations and have a higher average performance than females. The performance of female rats is stabilized by administration of estradiol benzoate (which induces pseudopregnancy) and by ovariectomy in contrast to controls. Administration of testosterone propionate markedly enhances the performance of ovariectomized female rats. The effect is dose dependent.

GENERAL INTRODUCTION

A dog's capacity to respond to odor depends partly on its ability to detect the odor, discriminate it from others and identify its attractive or aversive properties. These attributes are partially interdependent. Here, however, we are primarily concerned with detection. We are secondarily concerned with preference only in so far as it may provide a means of evaluating the capacity to detect odors, and we ignore discrimination.

Our major aims were to define minimum techniques sufficient to group dogs according to their ability to learn an odor detection task (Part I) and secondly to develop a quantitative method for testing the capacity of dogs to detect odors under more rigorously controlled experimental conditions (Part II). A final aim -- partly realized in the period covered by this report -- was to use this latter technique to investigate factors influencing the ability to detect odors (Part II). Supplementary studies with rats described in Part III cover this problem in relation to hormonal factors.

The studies described in Parts I and III exemplify the so-called "hypothetical-deductive" approach in which the answer to an initial question provides information used to formulate a second testable hypothesis and so on to form a logically connected series of experiments. The studies covered in Part II follow this logic only in part. Thus, while their focus is on developing a sensitive and accurate method of measuring odor detectability in the dog, the central problem lies more in stimulus delivery, measurement and control. This factor, together with the intelligence and extreme sensitivity of the dog to odors, places severe stress on adequate

control of the experimental situation.

However, there is one question which is common to all the dog studies reported here, and that is the identification and characterization of individual differences in performance (in both detection and preference behavior). In the case of detection thresholds, marked individual differences would be expected on the basis of previous studies. Thus Moulton et al (1960) found that thresholds for butyric acid in two labradors differed by a factor in excess of ten. In the case of preference behavior only anecdotal evidence seems to exist. Individual differences due to genetially controlled factors can be minimized by using littermates. This approach has been exploited in the studies reported in Part II. In Part I, however, we were concerned with extracting subjects from a relatively unselected population of German shepherds in order to increase the probability that we would be investigating a broader and more representative range of performance.

Previous studies on dogs have been reviewed by Moulton et al (1960) and by Benjamin et al (1965).

PART I

THE ASSESSMENT OF ODOR PREFERENCE OR AVOIDANCE IN DOGS AND ITS RELATION TO PERFORMANCE IN LEARNING AN ODOR DETECTION TASK

Introduction

A series of five experiments was run using two different types of apparatus. Each apparatus provided the dog with two water stations -- one associated with a test odorant and the other with a blank substance (having no odor for man). The idea was that if the dog found the test odorant attractive or aversive, it would tend to approach or avoid the drinking station associated with that odorant.

The first four experiments involved no training and were, in essence, modifications of the well-known two-bottle preference test. We used this te with the aim of deriving a means of grouping dogs according to degree and direction of preferences. This would identify individuals showing abnormal or low responsiveness. We also hoped to identify compounds particularly effective in eliciting strong preferences or aversions in dogs. For these reasons, the test stimuli selected were compounds (or compounds related to) those known from the literature to elicit unusual responses in dogs, as well as a constituent of dog anal gland secretions (valeric acid).

In the final experiment, the aim was to determine whether the rankings derived from the earlier study would show any correlation with a further measure based on the ability of dogs to learn an odor avoidance response.

Since the first three sets of experiments gave inconclusive results they will be summarized only briefly. Their chief value lies in demonstrating the evolution of the rationale behind the final experiment.

Experiments 1 and 2. Preference during restricted access to water.

The aim of these experiments was to determine whether significant information about preference behavior could be estimated by measuring water intake from a source in close proximity to an odorant in dogs having restricted access to water.

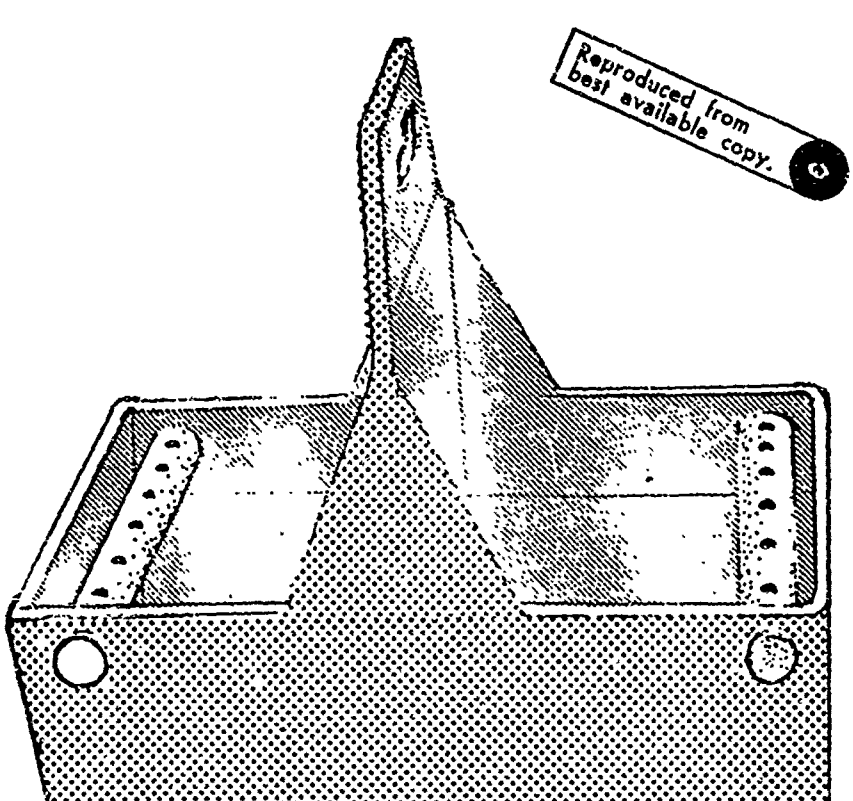


Fig. 1 Odor preference trough

Materials and Methods.

Two kinds of apparatus were used: a rectangular plastic box divided into two equal sized compartments by a vertical partition (Fig. 1) and a modification of the box shown in Fig. 2.

In the case of the plastic box, holes drilled in the side wall allow the insertion of two hollow metal cylinders so that each lies just under the rim of the wall opposite the vertical partition: one cylinder in each box. Glass wool placed in each tube was impregnated in one case with valeric acid diluted with methyelene chloride to 10^{-2} and in the other with methylene chloride alone. The compartments were filled to the same level with water. The relative positions of the odor and blank compartments could be reversed by rotating the box through 180°. This was done between trials where indicated by a randomly determined sequence.

In the case of the second apparatus, the dogs drank from steel bowls placed at the base of a wooden box divided vertically in its lower section. Each bowl was fed by siphon action from a calibrated 4 ℓ bottle in the upper part of the box as shown in Fig. 2. However, hinged metal doors and associated teflon tubes were not present in this experiment and odorant or distilled water was placed in a Petri dish beneath each bowl. A row of holes drilled just inside and around the rim of each drinking bowl allowed odor or water vapors to diffuse from the Petri dish to the vicinity of the water inside the bowls. Odorants used were butanol, ethyl propionate and pyridine.

In both sets of experiments, 5 females and one male (No. 6) German shepherds were used. They were placed on a restricted water intake and brought singly to the test apparatus by the handler. Each dog was allowed to drink from either the "blank" or odor-associated side until it was satiated. The relative positions of odor- and water-containing Petri dishes were

changed according to a randomly determined sequence. A dog's preference was determined by the volume of water drunk from each side, read directly from the calibration of the containing vessel.

Trials with the plastic box were conducted in an outdoor dog run, while those with the wooden test station were run in the laboratory.

Results and Discussion

The responses of dogs in trials with the plastic box apparatus appeared to be determined largely by place preferences and because of the high variance and inconsistent scores obtained, the experiments were discontinued at an early stage.

The results of experiments with the wooden box are summarized in Table I. Each figure is the aggregate total for a series of trials. The odorant most able to elicit marked preferences or aversions was butanol despite the fact that pyridine has a pungent odor that is more aversive for man. Thus 92 per cent of the volume of water drunk by dog 4 was on the blank side while 84 per cent of the water drunk by dog 1 was on the odor side. (Odor and blank appeared as frequently on the left as on the right.) For all but two dogs butanol appeared to be at least slightly aversive while the reverse held for ethyl propionate. With pyridine there was an equal division of preferences between odor and blank. However, because the number of trials was small we can reach no reliable conclusions about individual differences except to say they exist. What is more relevant is the rapidity with which animals tested (using either the plastic or wooden boxes) made decisions to drink from a given side. This suggests that the animals were so highly motivated to drink that the presence or absence of odor may have had little influence on their choice. In other words, place preferences may have severely attenuated or suppressed the expression of any odor preferences or aversions

DOG Number	ODORANT					
	Butanol		Ethyl Propionate		Pyridine	
	Odor	Blank	Odor	Blank	Odor	Blank
1	15 (85)	1 (16)	65 (53)	57 (47)	105 (41)	157 (59)
2	34 (41)	49 (59)	70 (49)	72 (51)	173 (55)	141 (45)
3	23 (33)	47 (67)	109 (53)	97 (47)	208 (53)	185 (47)
4	5 (8)	58 (92)	101 (54)	85 (46)	118 (50)	118 (50)
5	36 (58)	26 (42)	60 (31)	133 (69)	195 (45)	234 (55)
6	22 (43)	29 (57)	109 (57)	82 (43)	222 (59)	156 (41)
Total	134 (40)	203 (60)	514 (49)	526 (51)	1021 (51)	991 (49)

TABLE 1. Relative preferences of dogs for three odorants. The amounts of water drunk by each of the 6 dogs at each of the two stations (odor and blank) are expressed in cc x 10^{-2}. The equivalent scores expressed as percentages are shown in brackets. The dogs were water deprived prior to testing.

that existed. Consequently, it was decided to discontinue these trials in favor of another approach.

Experiment 3. Preference during continuous access to water: Single measure.

The results of the previous experiments demonstrated that thirsty dogs did not show consistent and reliable odor-related preference when given only restricted access to water. However, it is possible that such preferences might appear if dogs had continuous access to water -- the rationale being that thirst would no longer be the dominant variable determining preference. A further experiment was therefore conducted with the box used in Experiment 2 (i.e. lacking the doors shown in Fig. 2). In this case, however, one box was left permanently in each dog's runway, and the amount of water drunk from each flask was determined once daily.

Butanol and valeric acid were used as stimuli. Its position relative to a "blank" was changed according to a randomly determined sequence on a daily basis. Four female and one male German shepherd were tested.

The results of this experiment were again inconclusive - a high variance tended to obscure any underlying preference that might have existed. There was some evidence, however, that under conditions of continuous access to the water solutions, place preference did not play as significant a role in controlling the dog's behavior as it did in previous experiments. Consequently, it seemed advisable to retain this feature but to investigate further methods of reducing variance.

Experiment 4. Preference during continuous access to water: three measures of response

The results of experiment 3 suggested that measurement of water intake alone might not be a sufficient criterion to determine whether a dog was

behaving differentially towards the odor-associated station. Other possible criteria include the relative frequency with which the dog investigated the stations and the amount of time it spent drinking from, or sniffing around, them. A further difficulty with the last experiment lay in ensuring that the dog detected odor before making a choice. Finally, it was argued that if access to water required a more positive action, the dog might be more selective in its performance.

These considerations were translated into modifications of the basic preference box described previously.

Materials. Preference box (Fig. 2)

The modified box as shown in Fig. 2 has the following added features:

1. Sheet metal doors (16-1/4" x 8-1/2") were added so that although each bowl is visible from in front, a dog cannot drink without first pressing back the door with its snout.

2. The odorant was carried on glass wool which was in turn housed in a hollow teflon tube (6-1/2" x 1/2") pierced along one surface (parallel to the long axis) with a row of holes. An identical tube was filled with glass wool impregnated with the diluent. (This tube is referred to below as the "blank"). Each tube fits into a bracket at the base of the exterior surface of the door so that it is about level with the dog's muzzle.

3. Two systems to measure time and frequency were added. The first consists of two clocks: one above each drinking station. Each clock operates only when the door controlling access to the station is pressed back. Thus it serves to measure the amount of time a dog spends in the station. The second system consists of two counters: one within each station. Each is triggered by a mercury switch activated by the raising of the door. Their function is to measure the number of entries into the

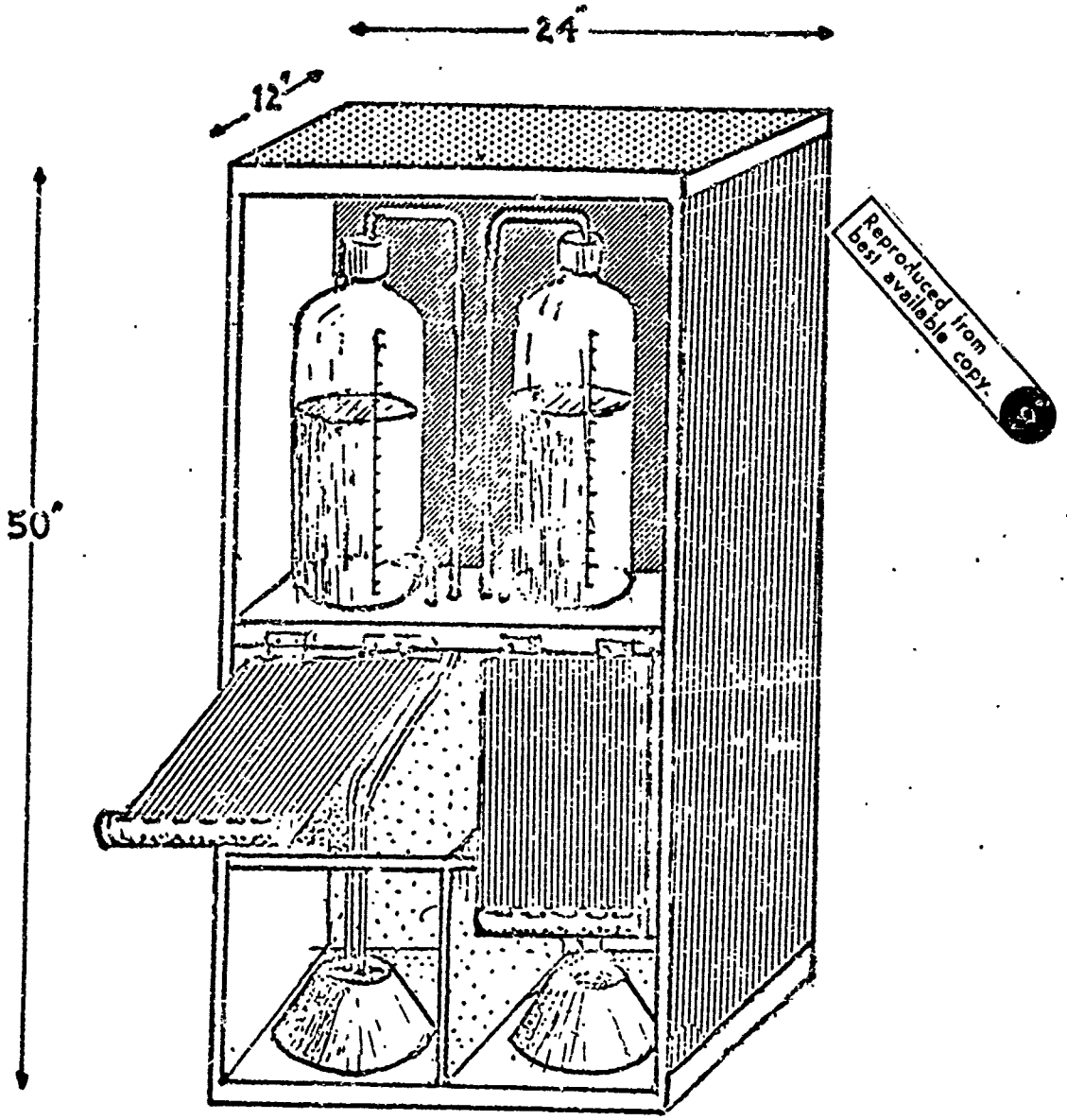


Fig. 2 Odor preference box

associated station. As in previous experiments the amount of water drunk was read directly from calibrated 4 l reservoir flasks.

Animals

The same four female and one male German shepherd were again used. No restrictions were placed on the amount of food or water they received.

Odorants

Benzyl benzoate was initially chosen as a blank diluent because it has little odor to man. However, when preliminary trials suggested that the dogs behaved differentially to this odor, it was used as an odorant. The other test odorants were butyric acid and valeric acid. The diluent for these compounds was methylene chloride, a highly volatile solvent that evaporates rapidly.

Methods

One box was placed at the end of each dog's run furthest from the kennel. Butyric and valeric acid were diluted by adding 1 cc of the odorant to 9 cc of methylene chloride, mixing this solution, drawing off 1 cc with a pipette, adding this to a further 9 cc of ethylene chloride and mixing again. Ten cc of the diluted odorant was sprinkled over glass wool which was inserted into the teflon tube and placed in the bracket at the base of the door of the preference box. The tube on the other door contained 10 cc of the diluent on glass wool. The diluent evaporated before trials began.

Because the diluent evaporated rather than remained to maintain the test odorant in solution, it is not strictly accurate to refer to the dilution as 10^{-2} saturation. However, this is a convenient way to refer to the amount of the odorant present.

Trials were run for 10 consecutive days per odorant. The relative positions of the odor and blank for each day were established by a randomly

determined sequence in which odor appeared as frequently on the left as on the right. Once a day, readings were taken from each station in each box of the amount of water drunk, number of times the door had been pushed back to gain access to the drinking bowl (number of entries), and the total time that the door remained open.

At the end of a 10 day period the accumulated total counts for each of these three indices of choice were determined for each dog. Consequently results could be expressed as three sets of paired measures. The pairs were then ranked 1:0 (odor > blank) or 0:1 (odor < blank). When these scores were summed across the three measures they gave an index of overall preference or response ranging from ratios of 3:0 to 0:3. Since the ratios cannot tie, the final preference estimate of response can be stated as a predominantely odor or blank preference.

Results and Discussion

To understand the basis for scoring, an example of a 10 day summary chart for each of the dogs is shown in Table 2.

This table shows that dog 1, for example, accumulated a higher total on the blank associated-station than on the odorized side by a ratio of 2:1. Consequently, this dog is considered to have shown an overall "preference" for the blank side.

In Table 3 the data expressed as rankings in Table 2 are shown along with comparable summaries for valeric acid and benzyl benzoate. From this data it is evident that the behavior of the single male dog (No. 6) did not differ significantly from that shown by the majority of the females in the case of benzyl benzoate and valeric acid, and there are no grounds for explaining any of the differences found in these studies in terms of sex differences.

DOG Number	Water Intake*		Number of Entries		Time**		Preference
	Odor	Blank	Odor	Blank	Odor	Blank	
1	135 0	147 1	146 0	203 1	32.08 1	29.02 0	Blank
2	200 1	188 0	276 1	266 0	25.33 1	22.26 0	Odor
3	145 0	237 1	184 0	195 1	30.47 0	47.52 1	Blank
4	142 1	140 0	296 1	281 0	24.50 0	27.08 1	Odor
6	110 0	132 1	171 0	216 1	34.40 1	31.46 0	Blank
Totals	782 2	844 3	1073 2	1161 3	147.58 3	158.14 2	Blank

TABLE 2. Accumulated totals for each index of response over a ten day period.

Odorant: Butyric acid (10^{-2}).

* Water intake expressed in cc x 10^{-2}.

** Time expressed in hrs . mins.

DOG Number	Water Intake*		Number of Entries		Time**		Preference
	Odor	Blank	Odor	Blank	Odor	Blank	
			BENZYL BENZOATE				
1	1	0	0	1	1	0	Odor
2	0	1	0	1	0	1	Blank
3	1	0	1	0	1	0	Odor
4	1	0	1	0	1	0	Odor
6	1	0	1	0	1	0	Odor
Total	4	1	3	2	4	1	Odor
			BUTYRIC ACID 10^{-2}				
1	0	1	0	1	1	0	Blank
2	1	0	1	0	1	0	Odor
3	0	1	0	1	0	1	Blank
4	1	0	1	0	1	0	Odor
6	0	1	0	1	1	0	Blank
Total	2	3	2	3	3	2	Blank
			V.LERIC ACID 10^{-2}				
1	0	1	0	1	0	1	Blank
2	0	1	1	0	1	0	Odor
3	0	1	0	1	1	0	Blank
4	0	1	0	1	0	1	Blank
6	1	0	0	1	0	1	Blank
Total	1	4	1	41	2	3	Blank

TABLE 3. Summary of preferences of 6 dogs for 3 odors as measured by three criteria of response. All dogs except No. 6 are females.

The following addition points emerge from Table 3. Firstly, with the exception of one female (No. 2) all animals showed the same preference for benzyl benzoate and the same aversion for valeric acid. In the case of butyric acid Female No. 4 as well as No. 2 showed a preference opposite from that of the remaining dogs. Secondly, no one measure alone shows this consistency. Thus, measures of water intake, for example, would not have identified dog No. 2 as differing from the other dogs in its performance. Thirdly, there is no evidence that measures derived from any two of the three possible pairings of indices show a significantly greater correlation than any two others. Finally, it is apparent that for a given odorant in any one dog, the direction of choice indicated by each of those measures frequently does not agree.

We have defined the direction of choice, derived by "pooling" the preferences obtained from the three indices of response, as the overall preference. (This is convenient but possibly misleading since in any given case it is not clear whether the dog was attracted to one side or repelled by the other.) The essential point to emerge from this study is that use of this overall preference measure makes it possible to identify consistently for each odor tested, a dog whose performance is the reverse of that shown by the majority. The question that now emerges is whether this finding predicts other aspects of this animal's capacities -- in particular, its ability to learn an odor detection task.

Experiment 5. Relative abilities of dogs to learn an odor detection task.

If the exceptional behavior of dog No. 2 in the previous experiment reflects reduced or distorted ability to detect odors, then its ability to learn an odor detection task might similarly be reduced, compared to that of

the other dogs. To test this hypothesis, and to determine whether dogs with unusual performance could be identified the dogs were trained to avoid a blank and drink from an odor-associated station.

Materials and Methods

The testing apparatus is shown in Fig. 3. It consists of two parts: a vertical test station and two entrance tunnels. The test station is in essence that described in Fig. 2. (Since no measurements of water intake, number of entries, or time were necessary, two drinking bowls behind swinging doors at the rear of the tunnel would have served equally well. The test station was used simply because it was available.) Each tunnel was constructed of sheet metal and an odorant or blank tube was fitted in a recess in its roof.

The tunnels led to the swinging doors of the test station and created a time delay preceding the intake of water. Available water was signalled by the absence of odor in that tunnel while the tunnel associated with odor led to a blocked door. The idea was that this arrangement would place greater emphasis on the detection of the odor cue, since a wrong choice meant a longer wait and a further walk between trials. It was also argued that the tunnels would serve to delineate the odor/blank contrast for the dog by minimizing the possibility of odor cross-contamination spreading from one station to the other and by providing a more extensive region of blank or odorized air.

The five German shepherds tested in the previous experiment (one male, four females) were again used here. They were first trained on valeric acid (10^{-2}) and then on a ionone. Thirsty dogs were initially allowed to enter the tunnels and drink from either side without odor or blank in position. This allowed them to habituate to the apparatus. During training they were deprived of water except for that received during and immediately after testing. The hinged door controlling access to water was blocked in position on

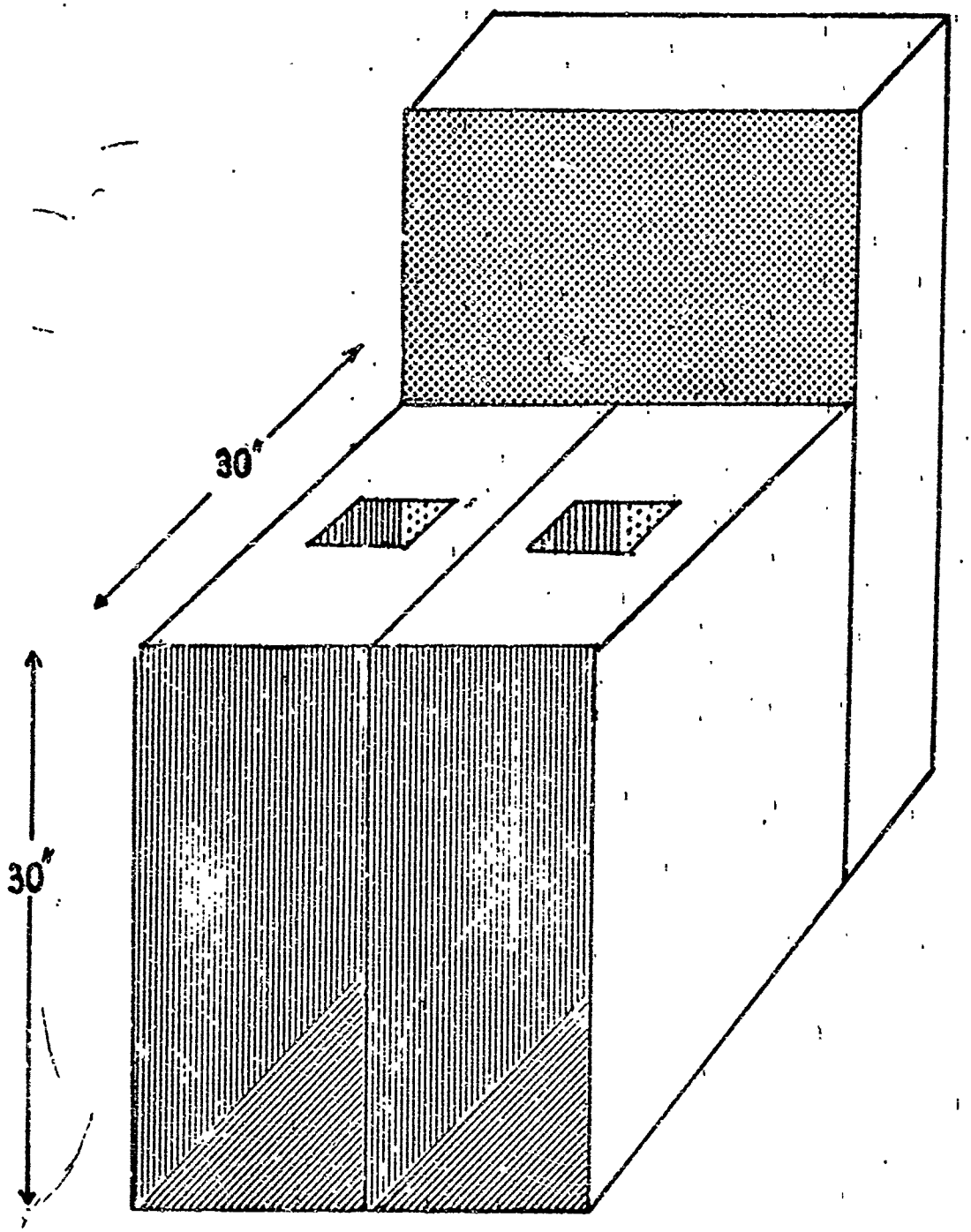


Fig. 3 Modified odor preference box

the side associated with the odor. Dogs were then individually given free access to the test apparatus. If the dog first went into the odorized tunnel and attempted to open the door leading to water, it was counted incorrect. It was counted correct if it first drank from the blank side. Dogs were given as many trials as they would complete, up to a maximum of 20 per day. In later stages of training this maximum was generally reached by all dogs.

Results

Figure 4 summarizes the performance of each dog on valeric acid while in Fig. 5 the means of session performances are plotted together with standard deviations.

The most striking feature of these results is that even the first session scores already identify one dog (No. 4) whose performance remained superior to that of the other dogs. Indeed, at a time when dog No. 4 first scored 100%, no other dog had attained scores higher than 64%. In contrast, the performance of dog No. 2 was inferior to that of the others and never exceeded 65%.

It might be argued that these divergencies in performances could be specific to valeric acid. We therefore ran further series of trials α ionone as the stimulus. This has a floral odor and in contrast to valeric acid, there is no reason to suspect that it might have a biological significance for the dog.

The results for α-ionone trials are summarized in Figs. 6 and 7. In this case, distinctions between dogs 2 and 4 and the remaining group are even more pronounced. Despite the previous experience, dog 2 did not appear to generalize its learning to the new odor immediately and chance scores were only exceeded on the trial session. Dog 4, in contrast, had already reached 100% by the fifth session.

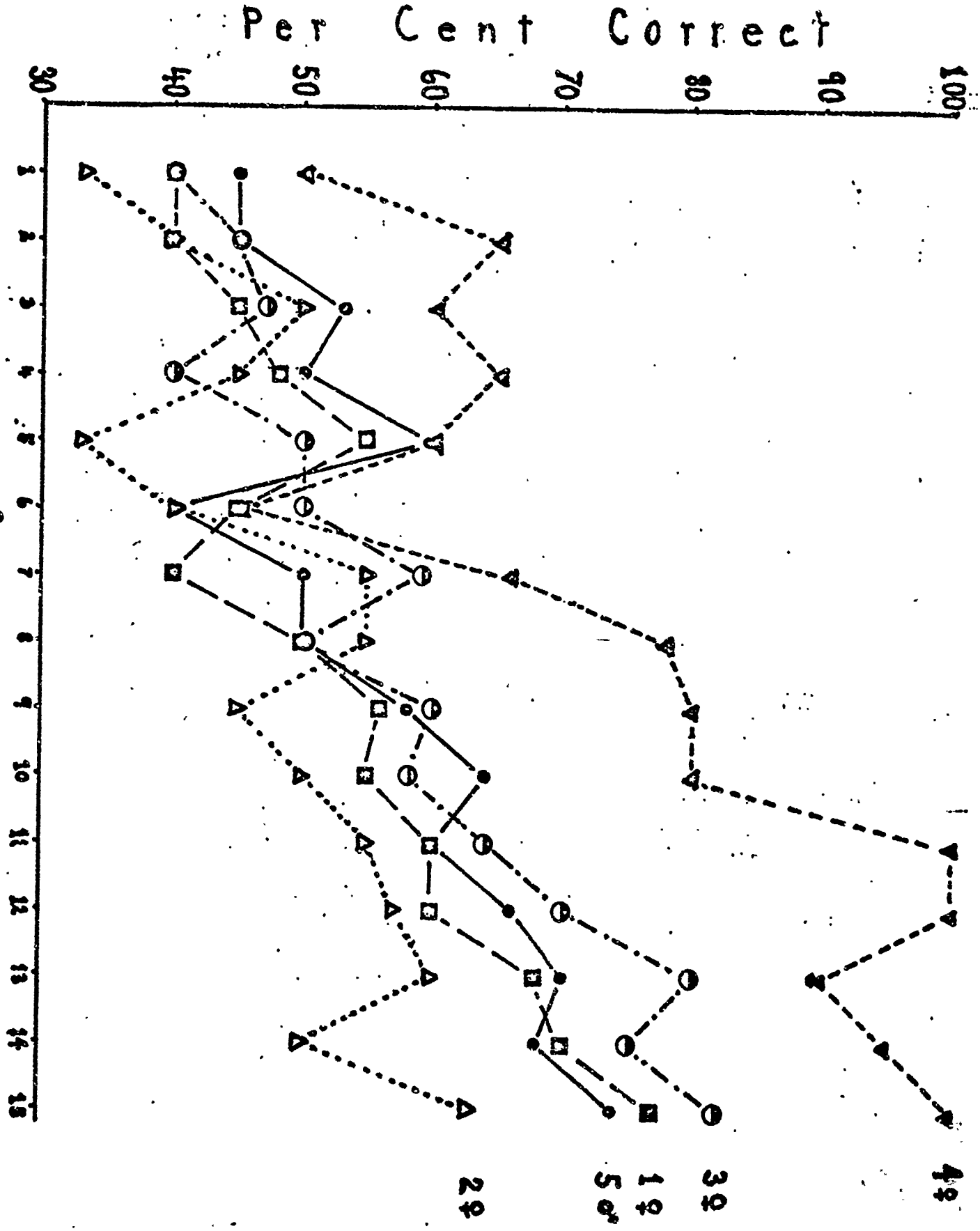


Fig. 4 Performance of five dogs during acquisition of valeric acid avoidance task. Chance score + 50% correct responses

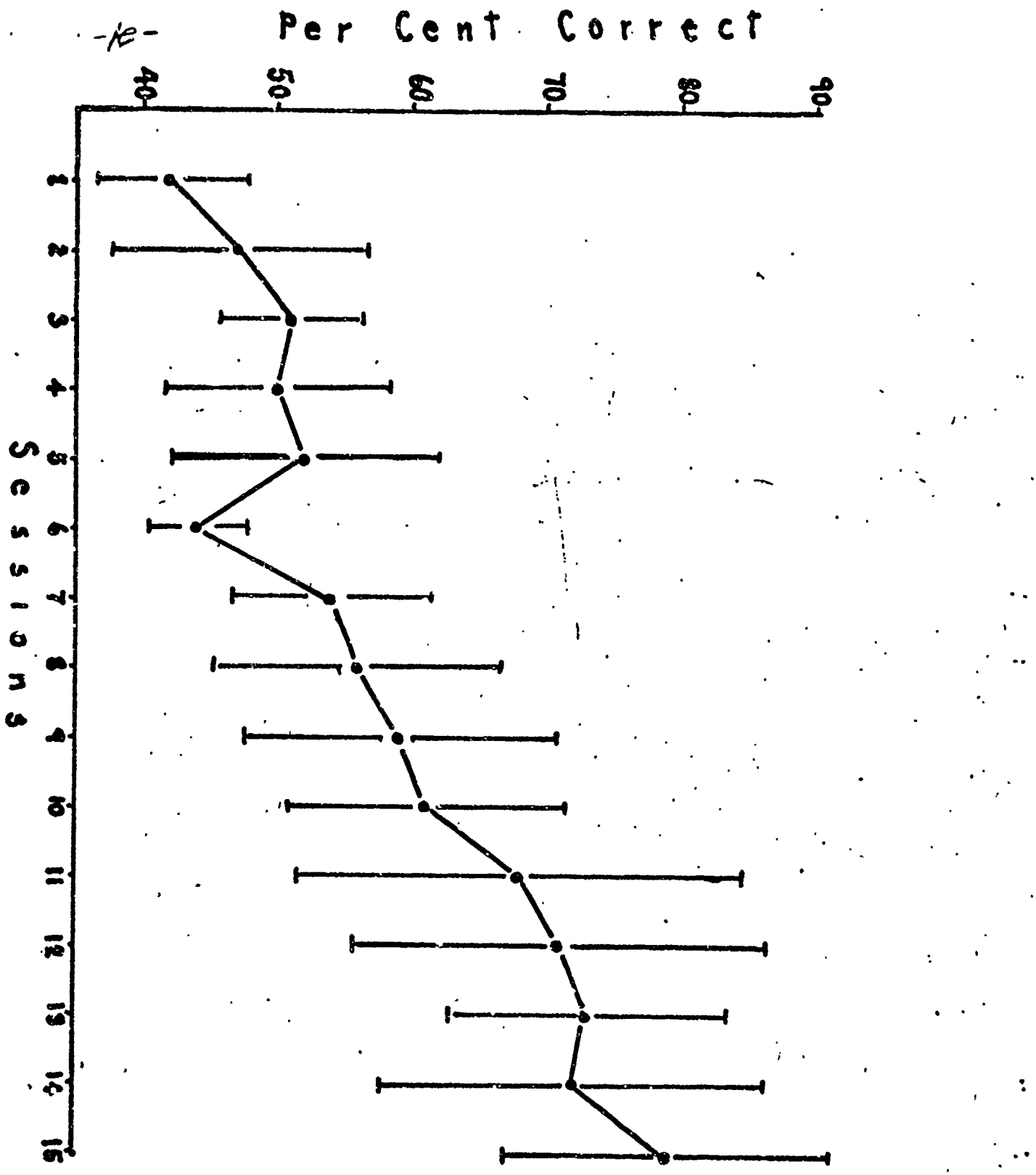


Fig. 5 Mean session scores for five dogs during acquisition of valeric acid avoidance task. Chance score = 50% correct responses

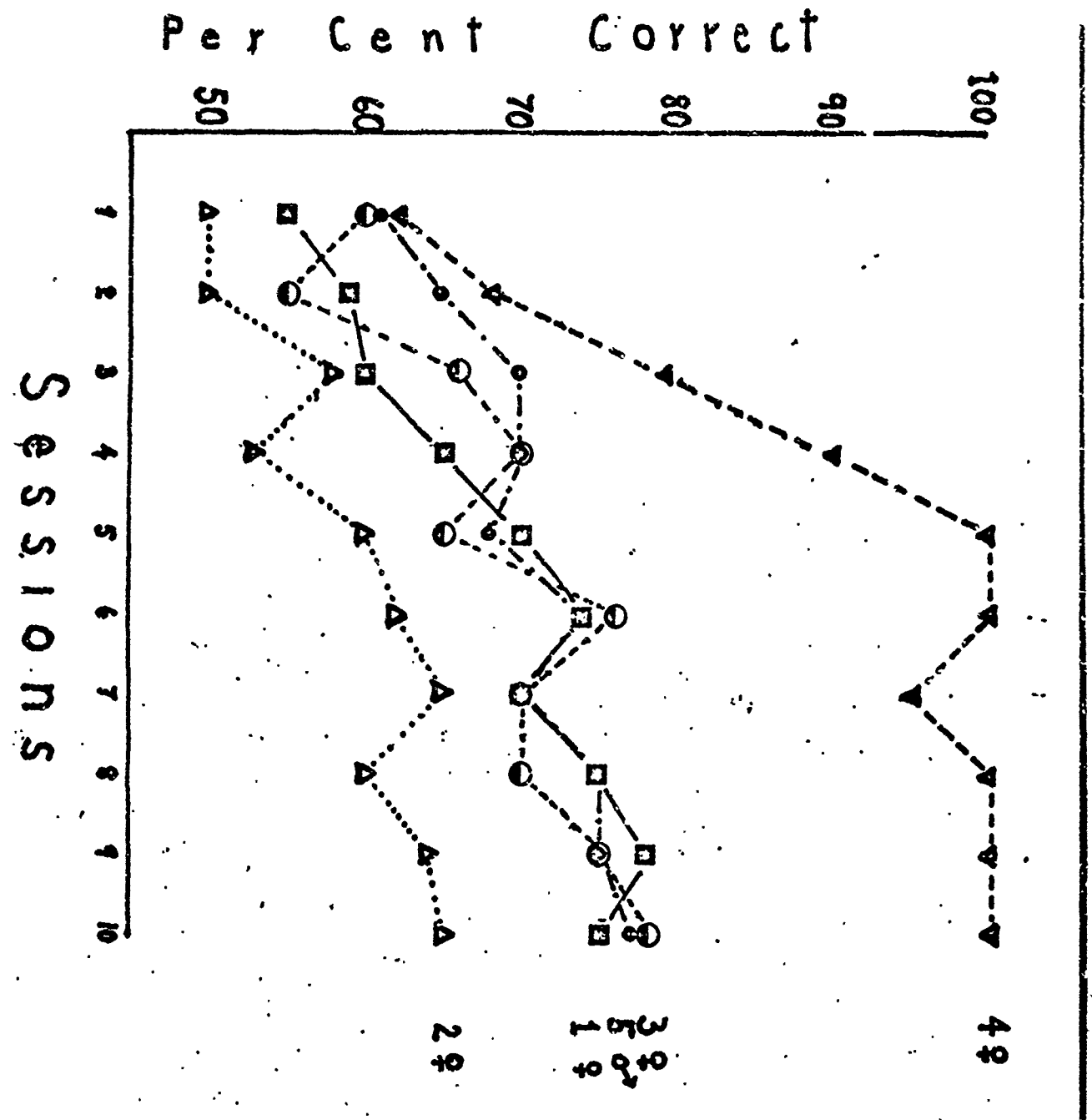


Fig. 6 Performance of five dogs during acquisition of alpha-ionone avoidance task. Chance score = 50% correct responses

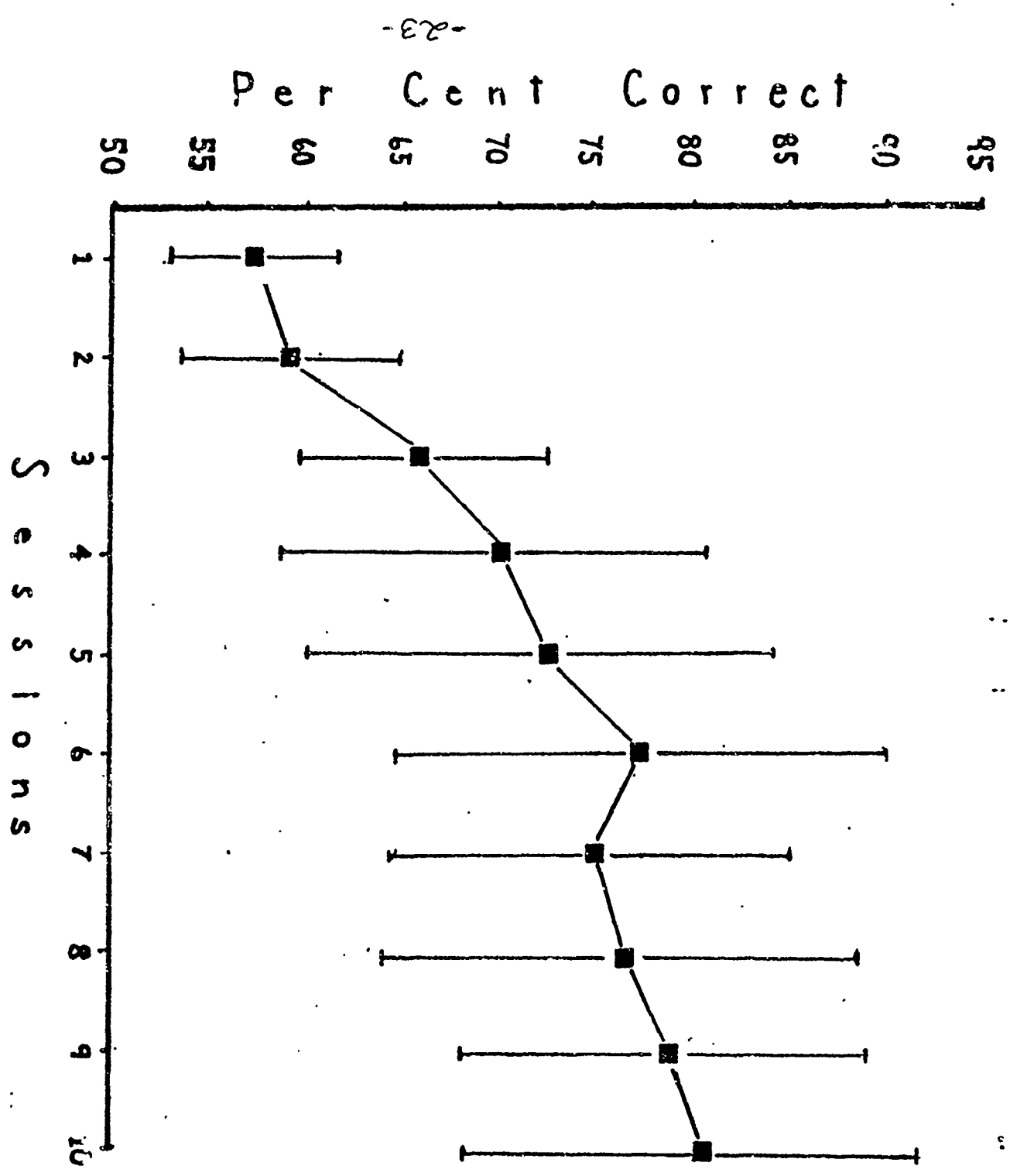


Fig. 7 Mean session scores for five dogs during acquisition of alpha-ionone avoidance task. Chance score = 50% correct

Comparing the mean performance on both odors shows that to reach the same score the dogs required 15 trials on valeric acid as opposed to 10 trials on α ionone. As a group then, the dogs did not generalize well to the second odor.

Discussion

The marked degree to which this technique segregates superior and inferior performing dogs suggests that it may have some value as a means of selecting dogs for breeding or other purposes. Without further studies, however, it cannot be concluded with absolute certainty that this performance is a measure of the dog's ability to detect odors, as opposed to a more generalized ability to learn sensory or other tasks.

This study also provided evidence of the ability of dogs to generalize from detection of valeric acid to detection of α ionone. The poor performance of the dogs in this respect contrasts with the ease with which dogs generalized from one fatty acid to another when trained to detect members of an homologous series of aliphatic acids (Moulton, 1960). This raises the possibility that generalization tests might provide a useful objective measure of the degree of similarity of different odors for the dog.

In the initial stages of training on valeric acid, scores of most dogs were below chance. This may be related to the findings of the previous study that all but one dog tended to avoid valeric acid (or prefer the blank side). However, the aberrant dog also scored below chance initially.

General Discussion

An aim of this series of studies was to identify techniques capable of grouping dogs according to their responses to odors. Part of this goal was to develop a method for evaluating odor preferences. But in addition, the idea

was to explore the possibility that such a technique -- requiring the minimum of supervision and no training of the dogs -- might also be helpful in predicting the more complex abilities of dogs to learn an odor detection task.

The preference testing apparatus finally evolved appears to satisfy at least some of the requirements for identifying individual differences. Furthermore, a correspondance was shown between the divergent preferences of one dog (No. 2) and its inferior ability not only to learn an odor detection task but also to generalize its learning experience from one odor to another. This suggests that a further goal of the study might also have been realized -- namely, to identify a relatively simple preference test that could be used to predict performance in an odor detection task. It should be emphasized, however, that since only a few subjects were involved and the number of trials was limited, this conclusion must be viewed cautiously. Nevertheless, the results are sufficiently encouraging as to suggest that further investigation would be warranted.

But whatever reservations may exist concerning the correlation between odor preference and detecting abilities there can be little doubt that one further aim of these studies was realized -- namely, to identify a method for selecting not only dogs with a marked inferior ability to learn an odor detection task but also those showing a superior performance. While this technique does require that dogs be trained, the apparatus is simple and reliable differences appear to emerge at an early stage of training.

A final point to note is that the above studies were conducted on German shepherds and the conclusions reached concerning odor preferences do not necessarily apply to other breeds. In fact, it is a common observation among those familiar with "working" dogs that some breeds--such as the Bluetick or Walker hound--more actively investigate the scent of small mammals than that of game birds--a trait sometimes claimed to be independent of training.

BIBLIOGRAPHY

Benjamin, R. M., Halpern, B. N., Moulton, D. G., and Mozell, M. M. (1965). The Chemical Senses. Ann. Rev. Psychol., 16, 380-416.

Moulton, D. G., Ashton, E. H., and Eayrs, J. T. (1960). Studies in olfactory acuity 4. Relative detectability of n-aliphatic acids by the dog. Animal Behavior, 8, 117-128.

TABLE II .

THE MEASUREMENT OF THE DETECTABILITY OF DIFFERENT

ODOR CONCENTRATIONS IN THE DOG

INTRODUCTION

Much confusion and controversy exist concerning the dog's olfactory powers and anecdotal accounts of extraordinary performances in tracking, and in detection of personnel and hidden objects abound. In some instances, it is not evident that only odor cues are involved, but even when this is established it is often not clear what are the relative roles of training, intelligence, and inherent sensitivity of the olfactory systems, or of detection as opposed to discrimination. Thus, because a well-trained dog can discriminate between the odors of identical twins (Kalmus, 1955), it does not follow that its ability to detect odors is superior to that of man. Consequently, discrimination, localization and detection are attributes that require separate assessment.

But even in relatively well-controlled investigations where detection rather than discrimination was clearly involved, marked discrepancies have arisen. Thus Neuhaus (1953) found that the dog's ability to detect butyric acid was in the order of 100 million times greater than man's, while Moulton et al (1960) found that these differences were more in the order of 100 to 1000. This is still a significant superiority but it is one that might result from the far greater natural training of dog in odor detection as compared to man and its more extensive central processing equipment. It is not entirely clear from Neuhaus' work that he eliminated all possible cues that could be used by the dog (e.g., auditory cues, flow differences between odor and air lines), and further information on this point is needed. To

derive such information, however, and to provide a sensitive technique that can be used to evaluate individual differences as well as factors that might influence odor detection, demands an adequate testing method.

A variety of methods for training animals to detect odors have, in fact, been described, ranging through classical and operant conditioning to conditioned suppression methods (see Moulton, 1973). However, the majority suffer from one or more disadvantages. For example, techniques involving shock may stress dogs to the point where an adequate performance is not possible (Becker et al, 1958), and activation of the sympathetic functions could, in any case, lead to abnormal threshold values. It also seems important to provide the animal with the opportunity to compare odor with no-odor situations and to correct its choice, if necessary, to obtain the most sensitive measure of response.

It is true that the technique described above (outlined in Part I, Experiment 5) does provide a method which satisfied these requirements. However, it does not allow for effective control of stimulus concentration or background odor. Achieving these and other controls requires a stable environment and a dynamic air dilution system capable of delivering a range of odorants in the extreme dilutions necessary to approach the absolute thresholds of detection in the dog.

In addition, the apparatus used in the odor avoidance study - although adequate for the purposes for which it was intended - was inefficient in terms of time required to obtain a unit of information. This time can be greatly reduced in an automated apparatus in which the dog can itself initiate each trial in a series of programmed trials.

THE APPARATUS AND ITS OPERATION

1. General features.

The apparatus shown in Figure 8 was developed from one described for use with rabbits (Moulton et al, 1970). However, it provides three rather than two choice points - a feature found effective in training opossums to discriminate odors and taste substances (Marshall, 1968; Marshall and Moulton, 1970). The encorporation of a delay preceding delivery of a reinforcement for correct choice was a concept also derived from the opossum studies.

In essence, it is a device for presenting dogs with a choice between air and odor streams of known concentration and flow rate. It provides conditions which minimize the spread of odor after its delivery to the choice point, its operation is automatic and the handler can see the dog but can neither be seen or heard by the dog.

It consists essentially of a main compartment with three smaller stimulus presentation compartments or bays, projecting anteriorly. Each bay has a one way glass window and a glass door separating it from the main compartment. With the exception of these glass surfaces the entire interior of the box is lined with aluminum foil-backed teflon sheeting. The outer framework of the box is constructed of wood and pressed hardboard. This box, together with an olfactometer, is housed in an environmental

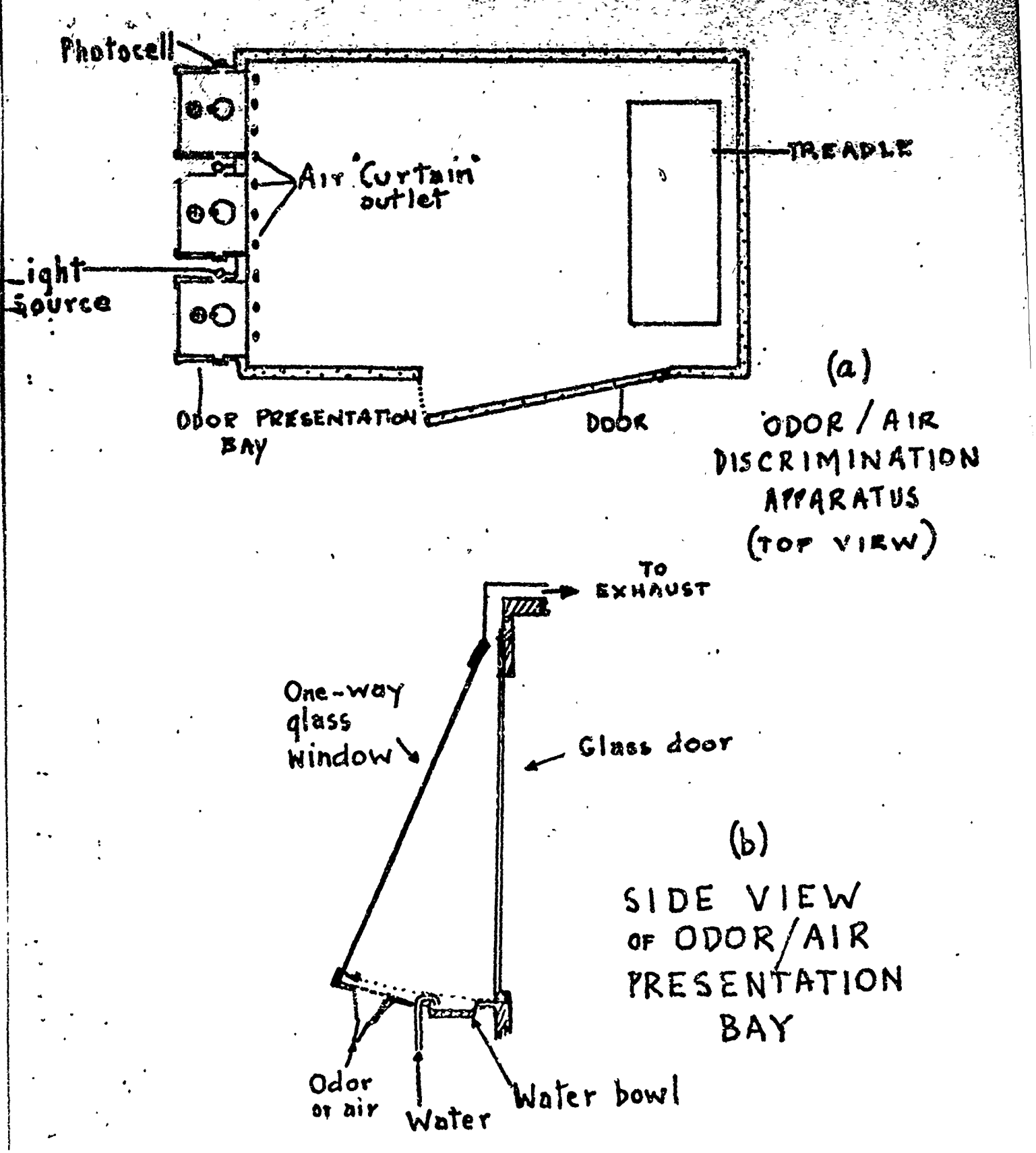


Fig. 8 Odor-choice apparatus for dogs

chamber maintained at a temperature of 34°.

The apparatus is controlled by a paper tape reader and system of solid state logic modules programmed to sequence odor presentation and control reward delivery through a series of teflon solenoid valves. An additional output function of this program is to control the motors operating access doors to each odor presentation bay. A printing recorder indicates positions of odor and of correct and incorrect responses. The prototype apparatus for this particular application of automated controls is described in Moulton et al (1970).

2. Discrimination box.

Access to the interior of the box is through a large door on the side of the box. On the floor near the rear of the main compartment is a treadle which initiates the sequence of one program cycle by opening the odor-air bay access doors. The roof in each bay and in the rear of the main compartment has exhaust outlets to a common duct leading out of the roof of the building. A curtain of purified air issues from a series of nozzles across the floor of the box beneath the bays. This curtain is drawn slowly upwards and backwards to the rear roof exhaust. It serves to evacuate the body and other ambient odors from the box.

The three detachable bays are identical. Each is triangular-shaped in profile, has a one-way glass panel on the front wall (to allow the dog to be observed during trials) and is separated

from the main compartment by a glass door hinged at the roof. When this door closes it blocks entrance from the main compartment to the bay. This allows the bay to be flushed between trials in relative isolation from the main compartment.

Odor or air from the olfactometer can be delivered to the floor of each bay by way of a perforated teflon disc about 6 cm in diameter, and is exhausted by the front roof duct. Water is delivered under solenoid control from a reservoir to a glass dish immediately to the rear of the teflon disc.

A beam of light is directed horizontally across each bay to a photocell. A dog breaks the beam when it inserts its head into the bay. The apparatus is programmed to allow a continuous sequence of odor and air delivery by way of teflon solenoids.

3. Olfactometer

Odorized air and air "blanks" are fed to the test box from an olfactometer. The following is the basic scheme of events:

Compressed air, delivered at a regulated pressure, is passed through a 4 l flask immersed in ethylene glycol cooled to -35° to provide an initial drying and reduction of impurities. It is then filtered through activated charcoal and silica gel and passed into a manifold with multiple outlets. One leads to a gas wash bottle where the air is bubbled through the test odorant (immersed in a water bath maintained at 23°). Odorized air leaving the wash bottle is then diluted up to six successive stages by

mixing with further fractions of air to give an output of known concentration and flow rate.

Because of the extremely low concentrations required in work with dogs, six separate dilution steps were considered necessary for maintaining accuracy. This was achieved by combining two, 3-stage olfactometers of different design that were originally operated separately. The first unit has a maximum dilution capability of one and one half log units ($10^{-1.5}$) per stage but in the experiments to be described it was run to deliver 10^{-1} per stage or a total dilution of 10^{-3} (of saturation at 23°). This dilution can bleed either directly to the test box or to the second unit of the olfactometer.

Each stage of the second unit can dilute by a factor of up to 10^{-2} to give a total dilution capability of 10^{-6} (or 10^{-10} for the entire olfactometer). Each stage of the second unit continuously delivers an output (by way of a three-way stopcock) either to the exhaust or to the test box. This permits concentrations to be changed rapidly during a session and ensures that equilibrium conditions obtain for each concentration up to the level of the three-way stopcock.

In addition to the air lines supplying the dilution stages two further lines supply the "blank" air line and an air "curtain" (described below).

The flow rates of odor and air to the bays are equalized by way of six needle valves, each line feeding three odor and three "blank" air solenoids. Bleed off valves in the odor and "blank" delivery lines (between the olfactometer and the solenoids) prevent excessive pressure build up during odor-air switching.

To clean the olfactometer and exposed surfaces of the box, the chamber temperature is raised to 60° C. Gas washing bottles containing alcohol and wrapped in heating tapes are substituted for the odorant bottles in the olfactometer. Air is then bubbled through them. The output of hot humidified air is fed into the box and exhausted and the procedure is continued for 24 hours.

4. Odorants

The odorant used was alpha-ionone - a compound with a floral odor. Exceptionally low thresholds have been reported for this compound in man and in the dog (Neuhaus, 1955). Because it absorbs strongly to glass and teflon surfaces, it creates special problems not only in cleaning the apparatus but also in establishing equilibrium conditions at low dilutions. For training purposes and for all trials involving concentrations of 10^{-6} and higher, the alpha-ionone used was obtained from K & K Laboratories, Inc. (Plainville, New York). The stated purity was 95-99%. For concentrations of 10^{-7} and less, the alpha-ionone used was obtained from Givaudan Corporation (Clifton, New Jersey). The stated purity was 99.2% (Irison alpha Lot # 3407-72). A gas liquid chromatographic analysis supplied by the manufacturers showed 5 minor peaks in addition to that of alpha-ionone.

SUBJECTS AND TRAINING PROCEDURE

1. Subjects

The subjects were four German shepherd bitches about two years old at the start of the experiments. Three were littermates. They were housed in kennels with extensive runs, and maintained on a standard laboratory diet.

2. Response Acquisition

Before training, dogs were deprived of water for 24 hours. Initially all the bay doors were closed except one. The odorant was delivered to this bay and the drinking dish filled with water. A dog was then allowed to enter the box. When it had learned to go immediately to the open bay and drink, the bay door was closed.

In the next stage, the dog was trained to activate the treadle either by walking, sitting or pressing down on it with its paw. As a result, two bay doors opened. One bay was still associated with the odorant but only "blank" air was delivered to the other. Training was achieved by closing the dog in the box and pairing its approach to the treadle with the sounding of a buzzer activated by the experimenter. The distance between dog and treadle was reduced until the dog was hitting the treadle. When the doors were open the dog was rewarded by the delivery of water only if it chose the bay associated with the odorant. The water was delivered when the dog's snout entered the bay. The positions of the odor and blank remained constant until the dog consistently went to the bay associated with odor. At this point the odor position was changed

over a series of trials according to a randomly-determined sequence.

When the dog's performance stabilized at a level indicating that it had learned the task it was given access to the third bay. Since this was also a "blank" air bay the dog's choice was now between two "blank" and one odor choice point. The position of the odor was still determined by a sequence which was random except that the same bay could not be associated with an odor for more than 3 times consecutively. Prior to this the intertrial interval was determined by the behavior of the dog. In subsequent training the intertrial interval was progressively increased to a 15 sec period. An attempt to activate the treadle within this interval had no effect.

3. Final shaping

The dog could insert its head into each bay and sample the air or odor as frequently as it chose, provided that it did not keep its snout in the bay for more than 3 seconds continuously. Thus there was adequate time for correction. However, when the photocell interruption was sustained for 3 seconds continuously a choice was registered.

If the dog selected the bay associated with the odorized air, 5 cc of water was delivered to the glass dish in the bay for a two second period. The dog was then allowed 5 seconds to drink the water. At the end of this period the doors of all the bays closed ejecting the dog from the bay. If, on the other hand, the

the dog selected a blank air bay all the bay doors closed simultaneously.

The dogs were trained until their performance reached a plateau (95% correct on 10 successive sessions where a session comprised 10-50 trials). The main door of the test box was closed throughout testing and the entire apparatus operated automatically.

TESTING PROCEDURE

The procedure used was that described above for the final stage of training with the following modifications. In initial experiments at higher concentrations of alpha-ionone, the period of sustained interruptions of the photocell beam that would result in the registration of a choice was three seconds (as in training). However, as the concentration was lowered, it was found necessary to increase the delay progressively to six seconds in order to allow for more prolonged sampling by the dog. The sequence of odor/air positions was also modified to eliminate the restriction of having no more than 3 successive odor presentations at the same bay.

A print-out was available for each trial of the position of the odorant, position of the dog's choice, number of photocell interruptions (including those less than the interval required to register a choice) as well as the total number of correct choices.

When changing from higher to lower concentrations, the effectiveness of the cleaning procedures was assessed by running "blank" controls. In these controls, a gas washing bottle containing water

was substituted for that containing alpha-ionone. The dogs were then tested, "correct" scores being given for the selection of the bay associated with the water. In some cases, scores above chance (33.3% correct responses) were obtained. It was therefore necessary to repeat the cleaning procedures (outlined above) until the chance scores were consistently achieved.

One possible source of error in the original design of the apparatus concerns the acute auditory sensitivity of the dog. When the programmer dictates a change in the relative positions of odor and air between one trial and the next a slight but audible click occurs due to the activation of certain of the odor/air solenoids. However, when successive trials involve no change the solenoids are silent thus providing a cue to the dog. To eliminate this possibility a duplicate bank of solenoids was installed and activated (by the programmer) between those trials that involved no change in position. They were not connected into the odor/air lines.

RESULTS

1. Flow rate discrimination

During one series of blank trials three of the four dogs tested scored significantly above chance. Since such anomalies had previously been traced to contamination of the odor lines, the olfactometer was recleaned. Despite this, the dogs continued to score above chance. It was finally established that, due to the introduction of an error in flow meter settings, the flow in

the normally odorized air line exceeded that of the blank air flow. Further testing revealed that the dogs were able to detect the difference between 8 and 9 l/min of air but not between 8.5 and 9 l/min.

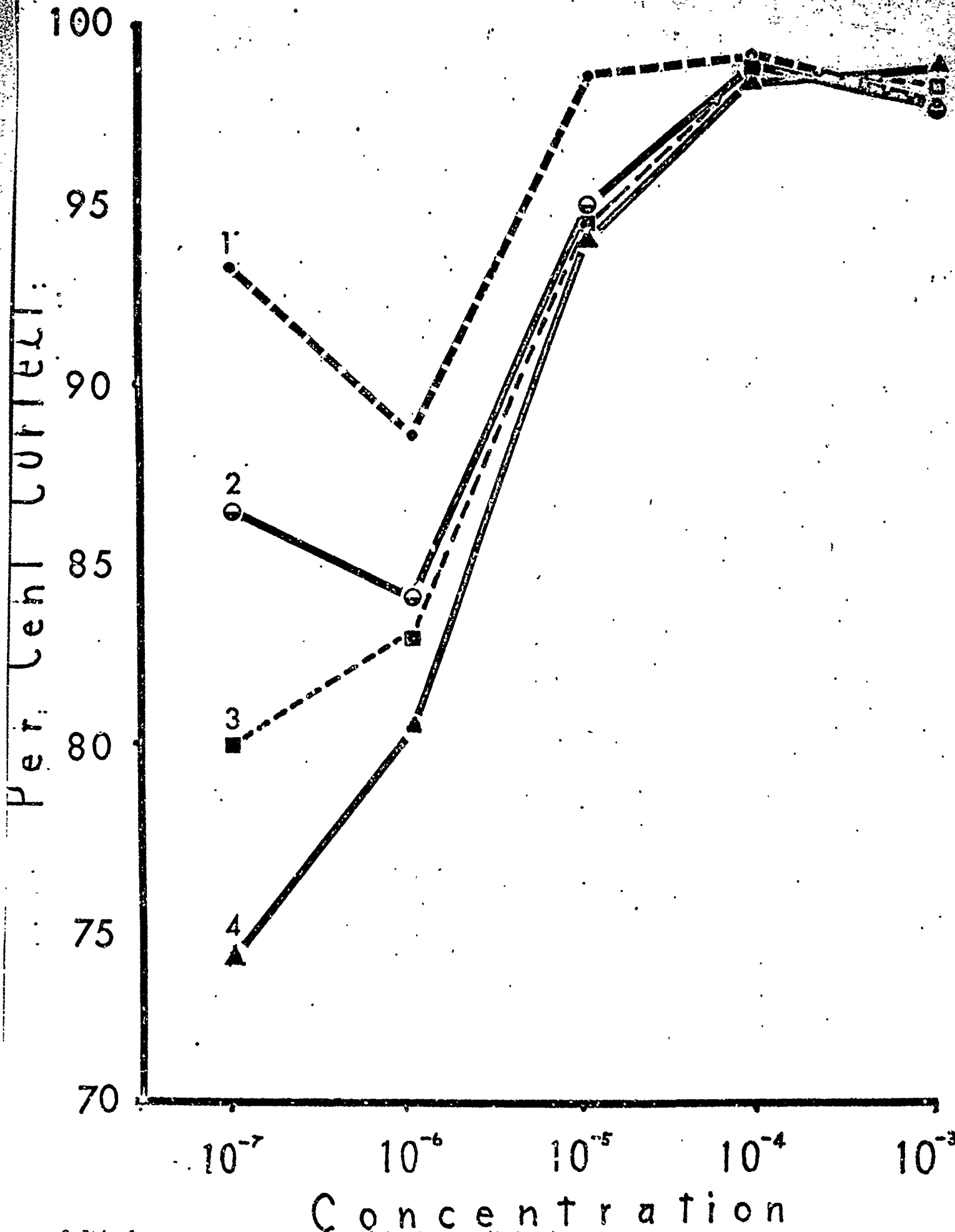
In other words, the Weber fraction (or just noticeable difference) defined by the ratio $\frac{\Delta I}{I}$ (where I is the intensity of the stimulus) is between 0.12 and 0.06 at this rate of flow. Weber fractions of less than 0.1 have been reported for touch, audition and vision but are higher for olfaction.

In subsequent trials, periodic checking of the flow settings prevented the recurrence of this source of error. The flow used in all definitive trials was 7.8 l/min.

2. Stimulus - response relations for alpha-ionone

The stimulus-response relations for alpha-ionone are shown in Figure 9. The asymptote of the curve falls at about 10^{-4} and at the lowest concentration represented (10^{-7}) the scores are still significantly above chance. However, preliminary evidence on 10^{-8} suggests that there may be a rapid fall to threshold near this concentration which would yield a total dynamic range in the order of four log units of concentration for at least certain of the dogs tested.

The data for 10^{-7} show an apparent reversal in the curve for two of the four dogs. There is also a marked divergence in the performance of the dogs at this level. In general, variance increases as concentration decreases, and seems to increase least for



. 9 Stimulus response curves for alpha-ionone (4 dogs). Chance score = 33.3%. All dilutions are relative to air saturated with alpha-ionone at 23°C.

the dog (No. 1) showing the best performance at lower concentrations. This dog, together with the next best performer (No. 2) did not actively seek human praise as did the other dogs, and they spent much time in sniffing--particularly when introduced into new surroundings.

DISCUSSION

The results establish that the method is an effective one for testing dogs on odor-detection tasks and for ranking their performance. The blank trials (given when concentrations were changed) demonstrate that only odor cues were determining the dogs' performance.

This experiment also shows what was evident in Experiment No. IV (Part I), that the dog showing the best performance is also the dog showing the greatest consistency.

The stimulus-response curve for alpha-ionone was not completed at the time this report was written, and it is intended to conclude this experiment by establishing threshold and then ascending through the concentrations to 10^{-3}. This may conceivably modify the conclusion to be drawn concerning the form of the curve, etc. However, informal tests on four human subjects in the same apparatus suggest that the sensitivity of the dog to alpha-ionone is probably at least 1,000 times as great as that of the untrained human subject although possibly this gap could be reduced by training the human subjects. This difference does not presently agree with the

much greater discrepancy between man and dog reported by Neuhaus (1954).

BIBLIOGRAPHY

Becker, F., Markee, J.E. & King, J.E. (1967). Studies on olfactory acuity in dogs. 1. Discriminatory behaviour in problem box situations. Brit. J. Animal Behav. 5, 94-103.

Kalmus, H. (1955). The discrimination by the nose of the dog of individual human odours, and in particular the odour of twins. Brit. J. Anim. Behav., 3, 25-31.

Marshall, D.A. (1968). A comparative study of neural coding in gustation. Physiol. Behav., 3, 1-15.

Marshall, D.A. & Moulton, D.G. (1969). Olfactory discrimination and stimulus coding in the opossum. Fed. Proc., 28, 275.

Moulton, D.G. (1973). The use of animals in olfactory research. In: Gay, W.I. (editor). Methods in Animal Experimentation IV, Academic Press, N.Y. (in press).

Moulton, D.G., Ashton, E.H. & Eayrs, J.T. (1960). Studies in olfactory acuity IV. Detectability of n-aliphatic acids by the dog. Anim. Behav., 8, 117-128.

Moulton, D.G., Celebi, G., & Fink, R.P. (1970). Olfaction in mammals - two aspects: proliferation of cells in the olfactory epithelium and sensitivity to odours. In: Wolstenholme, G.E.W. & Knight, J. (editors), Ciba Foundation Symposium on Taste and Smell in Vertebrates. Churchill, London, 227-250.

Neuhaus, W. (1953). Über die Reichschärfe des Hundes für Fettsäuren, 2. vergl. Physiol., 35, 527-552.

Neuhaus, W. (1954). Die Reichschwellen des Hundes für Jonon und Äthylmercaptan und ihr Verhältnis zu anderen Reichschwellen bei Hund und Mensch. 2. Naturforsch., 9b, 560-567.

PART III

ALTERATIONS IN THE DETECTABILITY OF ODOR AS A FUNCTION OF HORMONAL LEVELS IN THE RAT

INTRODUCTION

Assuming that an animal is "attending" to or "focussing" its olfactory system on the detection of an odor, its performance may vary according to such factors as age, nutritional and endocrine status. Of these only endocrine status has received much attention and the existing evidence suggests that this may be an important variable.

In particular, it has been shown by Le Magnen (1952a) and confirmed, in essence by several others, that the ability of women to detect the odor of Exaltolide fluctuates during the course of the menstrual cycle - the lowest thresholds occurring at or about the day of ovulation (Cluzel, 1964; Vierling and Rock, 1967; Guerrier et al, 1969). Köster (1965, 1968), however, found that the phenomenon was not restricted to Exaltolide, but was also shown - to a lesser extent - for xylene. The effect was related to the duration of the cycle: women with short cycles tended to be more sensitive to the odor in the 13 day period following menstruation than in that preceding it. The reverse situation occurred in women with longer cycles.

In contrast to these reports Klocek (1961) found no significant fluctuations in thresholds for Exaltolide during the menstrual cycle, while Schneider and Wolf (1955) found only elevated thresholds for citral during menstruation. In addition, Le Magnen (1952b) could demonstrate no cyclic variation in the rat's sensitivity to odors during the oestrus cycle. However, he did find significant

differences in performance between hormone treated, castrated and normal rats.

Despite these inconsistencies we can conclude that women's thresholds for at least two odorants show cyclic variations. The variations correlate with alterations in hormonal levels occurring during the ovarian cycle. No similar phenomenon seems to have been demonstrated in other species and no attempts have been made by experimental modifications to determine the relative role of different hormones in controlling this effect, or the level(s) at which the effect acts.

While training rats to detect the odor of cyclopentanone, we noticed that females showed marked fluctuations in performance correlated with the estrous cycle (Pietras and Moulton, 1969). To confirm this effect we have extended these studies by comparing the performance of males and females on several odorants. We have also attempted to gain further insight into the hormonal factors controlling variations by experimentally inducing different hormonal states in females. This paper briefly summarizes the main findings. Part of this work has been reported by Moulton & Pietras (in press).

METHODS

The male and female Long-Evans hooded rats used were about three months old and weighed 200-250 g at the start of the training trials. They were group 2-3 to a cage (sexes separated), and placed in a controlled 14/10 hours light/dark cycle. Food intake was restricted but rats had access to water for only 30 minutes follow-

ing testing. Only females with a regular 4 day cycle were used.

An air dilution olfactometer delivered cyclopentanone, eugenol and alpha-ionone at a concentration of 10^{-3} of saturation at 25 $\pm$ 1 c, and Exaltolide at an unknown concentration assumed to be at or near saturation at 25 $\pm$ 1°.

Air, filtered through silica gel and activated charcoal, was fed to an olfactometer. One stream was saturated with the test odorant (or, in the case of Exaltolide, passed over filter pap coils impregnated with the compound). The second stream diluted the first when necessary to a known concentration while a third stream was used as a "blank" air line and wash. The second and third streams each flowed at 600 cc/min. and were fed to the odor-choice apparatus.

The odor-choice apparatus is similar to that described for use with rabbits by Moulton et al (1970) and for the dog in Part II above. In brief, it consists of a main compartment flushed continuously with filtered air delivered to paired nozzels at the front floor end (Figure 10). At this end there are also two vertical gas tunnels: odor flows through one and air through the other. Air/odor positions can be interchanged according to a pseudo-random Gellerman sequence. The thirsty rat can sample each flow in turn through a port cut into each tunnel. A spout projecting into the tunnel opposite each port delivers a water reward (0.1 cc) when required.

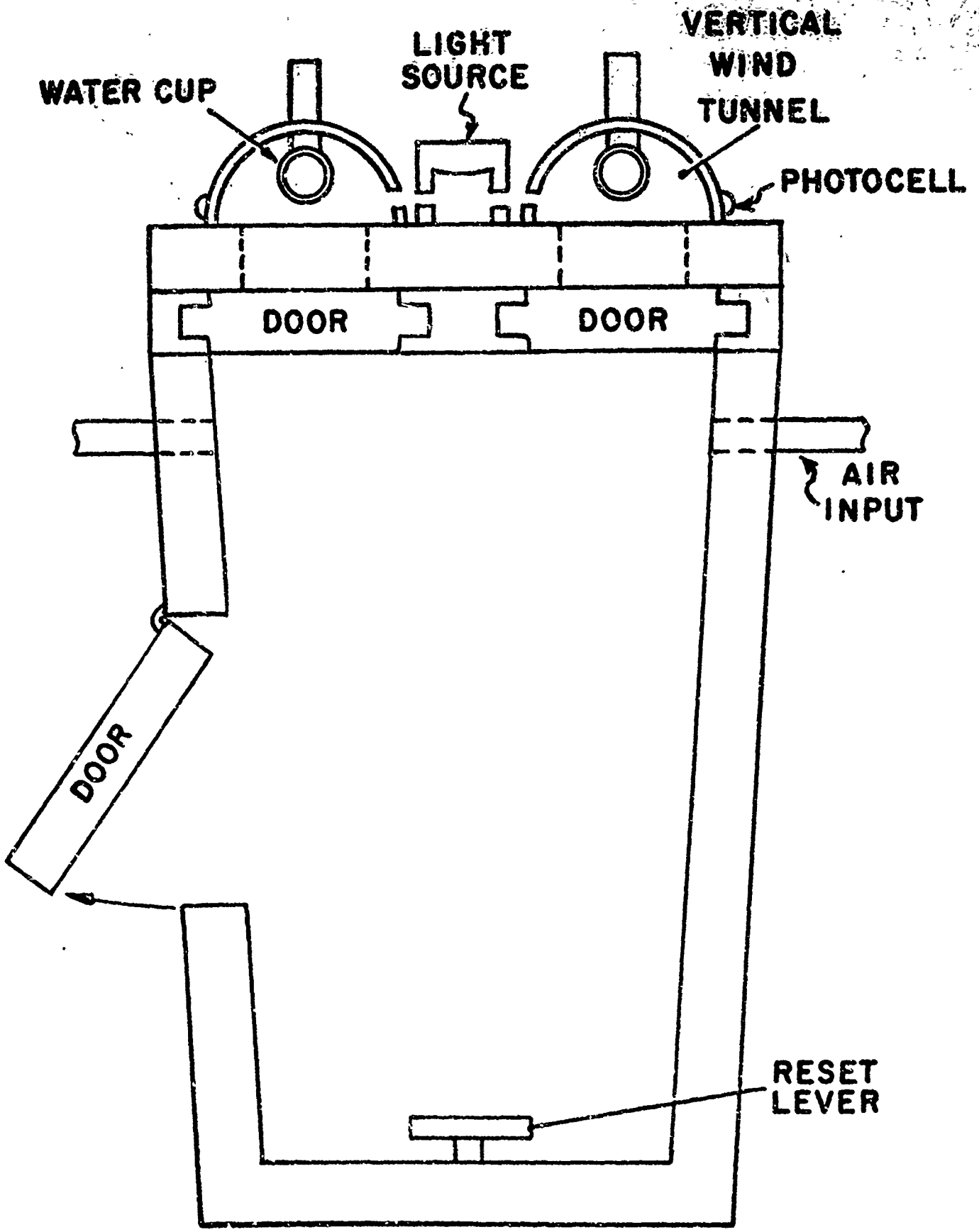


Fig. 10 Odor-choice apparatus for rats

The rat is required to detect the tunnel associated with the odor. It does so by interrupting for 5 seconds a beam of light directed horizontally (at the level of the port) at a photocell on the opposite side of each tunnel. If the rat selects the odorized tunnel it is allowed 15 seconds to drink the reward. At the end of this time teflon-clad doors descend in front of each port, ejecting the rat. If the rat selects blank air the doors descend immediately. In either case the doors remained closed until the next trial 60 seconds later.

Rats were trained on cyclopentanone (10^{-3} of saturation) on a 50:50 ratio of positive and negative forced trials (either blank or odorized air blocked). They were then given 10 free choice trials per day until they reached the criterion of 80 per cent correct choices on 5 successive sessions. Vaginal smears were taken at about the same time daily.

Differences in odor detection performance between experimental and control animals were assessed by X^2 and median tests. This allowed the significance of daily differences between the two groups with respect to odor response scores to be determined or permitted testing of the hypothesis that the two groups are from populations with different medians (Siegel, 1956). To determine whether scores from several successive days in the different groups were drawn from the same populations, the Friedman two-way analysis of variance was applied (Siegel, 1956). However, when the probability determined by this latter test appeared marginal, the former tests

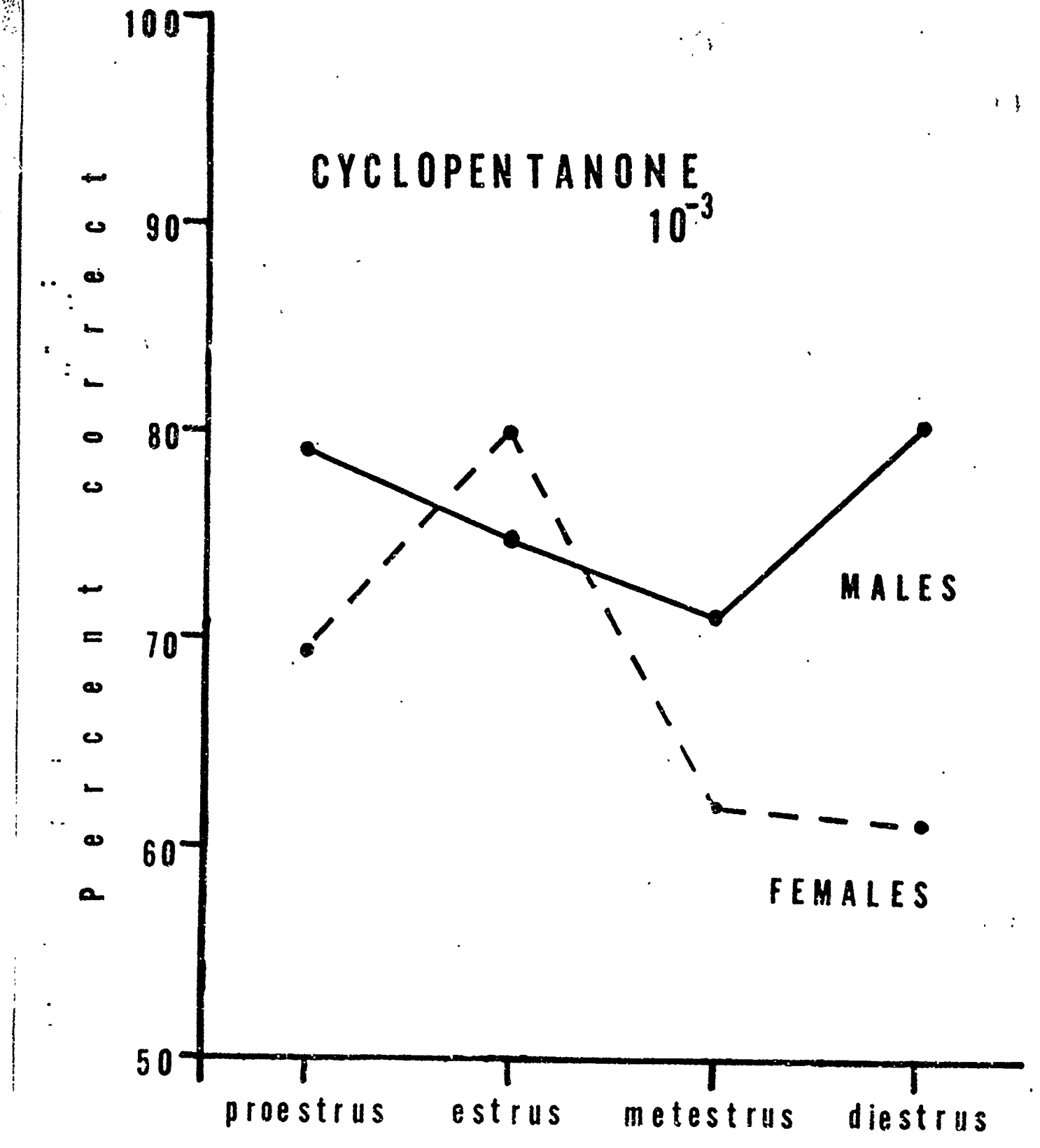


Fig. 11 Relative detectability of cyclopentanone by male and female rats over eight successive days

were applied to verify the level of significance in terms of daily variations in the scores between the groups.

RESULTS

Experiment 1 Relative detectibility of cyclopentanone by male and female rats over several successive days

The aim of this experiment was to determine whether there is any significant variation in the responsiveness of the females to cyclopentanone (at 10^{-3} of saturation) during the estrus cycle as compared with males during a comparable period.

A group of 7 males and 6 females was used. Each group was tested over an eight day period which corresponded to 2 complete estrus cycles. Since female estrus cycles were not synchronized, the daily performances of all rats for corresponding points in the estrus cycle (as determined by vaginal smears) have been summed and plotted together to facilitate display. The male's performance on the same days have been similarly added. These experiments were conducted in the early afternoon on each successive day.

The most striking feature to emerge from the comparison made in Figure 11 is the clear peaking of performance around the day of estrus in the female rats. The variation is significant ($p<.02$). In contrast, the performance of the male is stable and shows no statistically significant variation.

Figure 12 shows the performance of a single female as an example of an individual performance in which the fluctuations are particularly well defined.

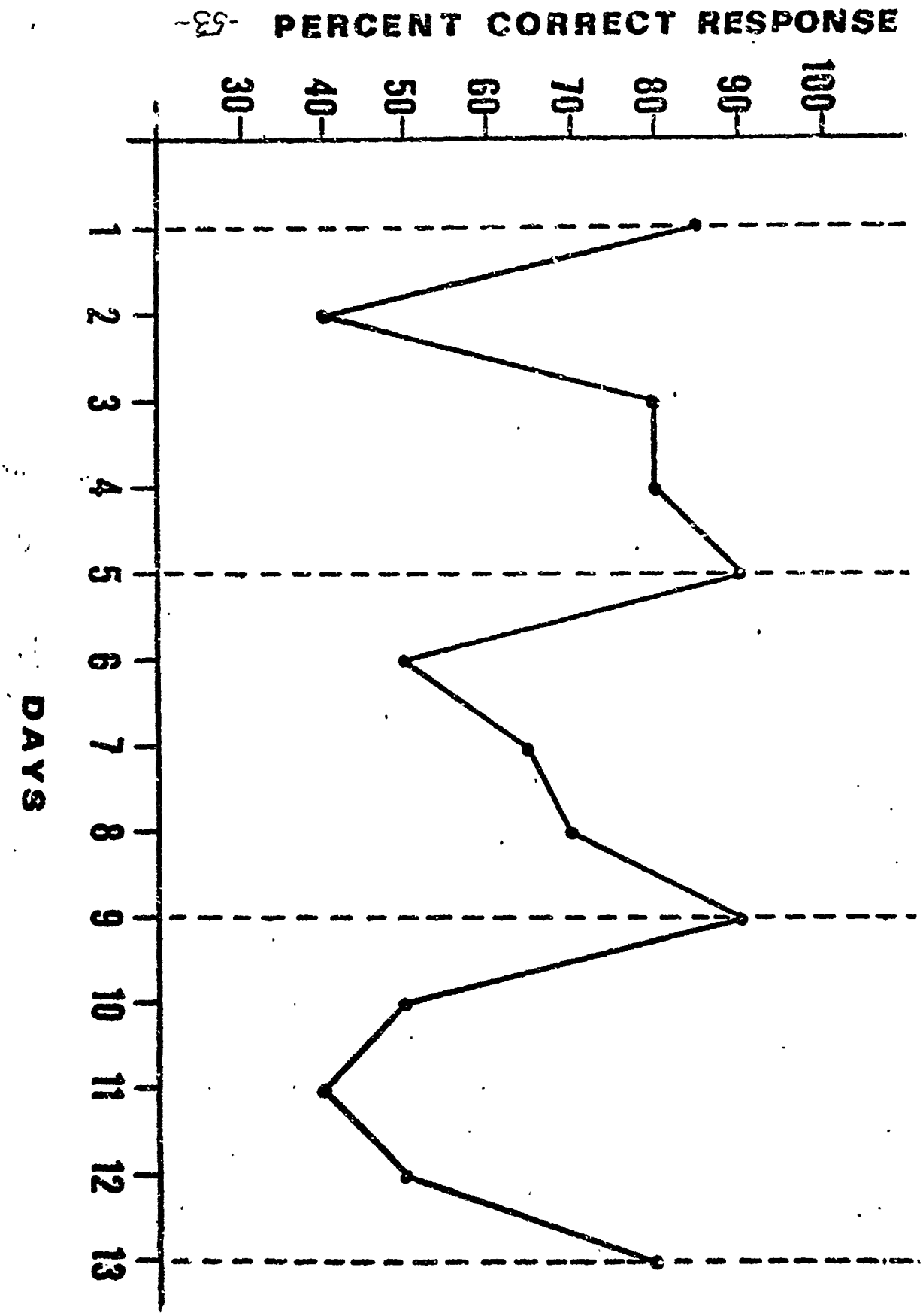


Fig. 12 Fluctuations in the performance of a female rat detecting cyclopentanone (10^{-3}) over the course of the estrus cycle

Variations in the responsiveness to cyclopentanone, eugenol, alpha ionone and exaltolide as a function of the estrus cycle.

The previous experiment showed that female rats vary in their responsiveness to cyclopentanone during the estrus cycle. To examine the generality of this phenomenon, three other odorants were investigated. All but one were presented at concentrations of 10^{-3}.

Six rats were tested in the early afternoon over a period of eight days - again corresponding to two estrus cycles - and given 480 trials to each odor. As can be seen from Figure 13, the peak performance for all odors occurs around the time of estrus. In all cases but one, the variability is significant (cyclopentanone: $p<.01$; eugenol: $p< .001$, $p<.01$; alpha ionone: $p< .001$, $p< .01$). In the case of Exaltolide, however, the variation is not significant, but the trend is in the same direction as that of the other odors tested.

Relative variations in responsiveness of male and female rats to eugenol and alpha ionone

Having shown variations in the response of females to a range of representative odors, it seemed important to know whether male rats also showed significant variations in response to odors. Data were derived from a group of males for comparison with the corresponding data obtained for females in the previous experiment. One group of seven males and another with six females received 640 trials for each of three different odorants over a period of 8 days or two full estrus cycles.

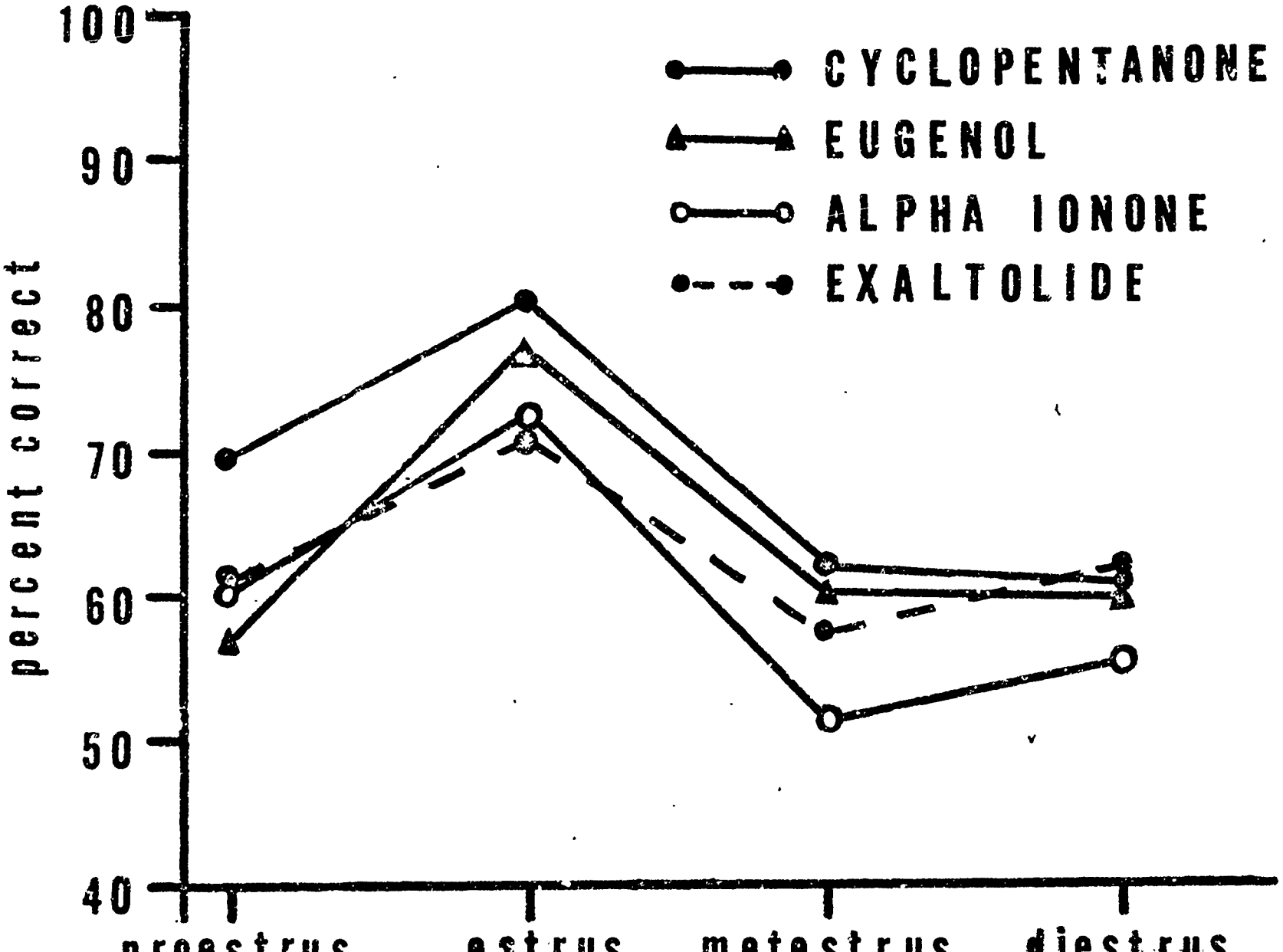


Fig. 13 Variations in the rat's responsiveness to four odors during the course of the estrus cycle

The results derived from the summed data of 1,920 trials per group indicate that the male rats do not differ significantly in their performance from day-to-day contrast to the highly significant variations shown by the females during the estrus cycle ($p < .01$). This is consistent with the data shown in Figure 11.

Influence of estradiol benzoate and of ovariectomy on the performance of female rats on an odor detection task (Figures 14 and 15)

If the variations observed in the performance of the females are in fact determined by ovarian hormone levels, experimental procedures which alter ovarian output should also be expected to affect performance on an odor detection task.

Six female rats were given a single injection of 5.0 µg estradiol benzoate/ 0.1 ml sesame oil on the day of cornification. All of these rats showed delayed ovulations as determined by vaginal smears and a prolonged vaginal diestrus lasting up to three weeks. These results are in agreement with those of previous workers (Gilmore and McDonald, 1969). [By acting to suppress LH secretion and induce prolactin release (Rothchild and Schwartz, 1965), the estrogens effect the maintenance of the new corpora lutea for periods of 14-19 days, and, thereby, induce a pregnant state (Gilmore and McDonald, 1969)]. The quantity of estrogen injected is not greatly beyond the physiological range (Gilmore and McDonald, 1969), but the generation of extraneous metabolic breakdown products cannot be excluded. A control group of six female rats was given

a single injection of 0.1 ml sesame oil on the day of cornification prior to these experiments. Each group was tested in the early afternoon in 720 trials extending over a 12 day period on cyclopentanone (10^{-3}).

In a further experiment to test the above hypothesis, 8 rats were ovariectomized under equithesin anesthesia to eliminate the influence of all hormones produced by the ovaries (Zarrow et al., 1964). These animals were allowed two weeks to recover from the operation in order to allow enough time for regression of residual sexual organs. A control group of 3 rats was sham operated. Both groups were tested in the early afternoon over a period of 8 days on cyclopentanone (10^{-3}). Each animal was subjected to 10 trials/day.

The results of the first experiment show that the induction of pseudopregnancy (PSP) not only eliminates fluctuations in performance of female rats but markedly depresses it almost to the chance level (Figure 14). In contrast, the fluctuations in the performance of the control group are depressed to a comparable level only on the afternoon of the proestrus day, the only point at which the differences between the groups are not statistically significant. The performance of the control group is significantly greater than the PSP group at the corresponding estrus days ($p<.0005$), maetestrus days ($p< .005$) and diestrus days ($p<.005$).

In the second experiment, the ovariectomized females attained a higher overall performance than the control group but the day-to-day differences were not significant (Figure 15). However, the

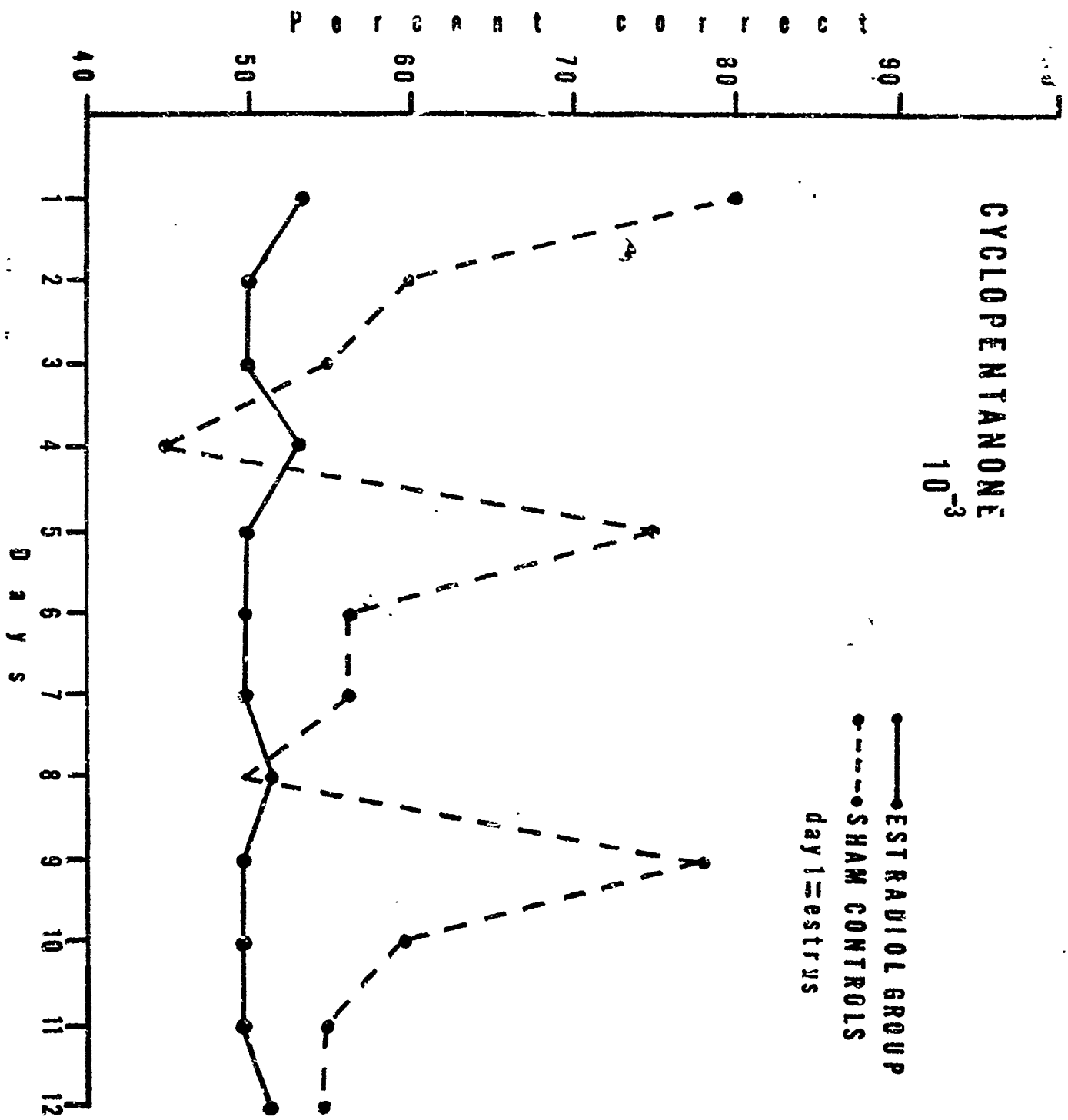


Fig. 14 Influence of pseudopregnancy on the responsiveness of rats to cyclopentanone (10^{-3})

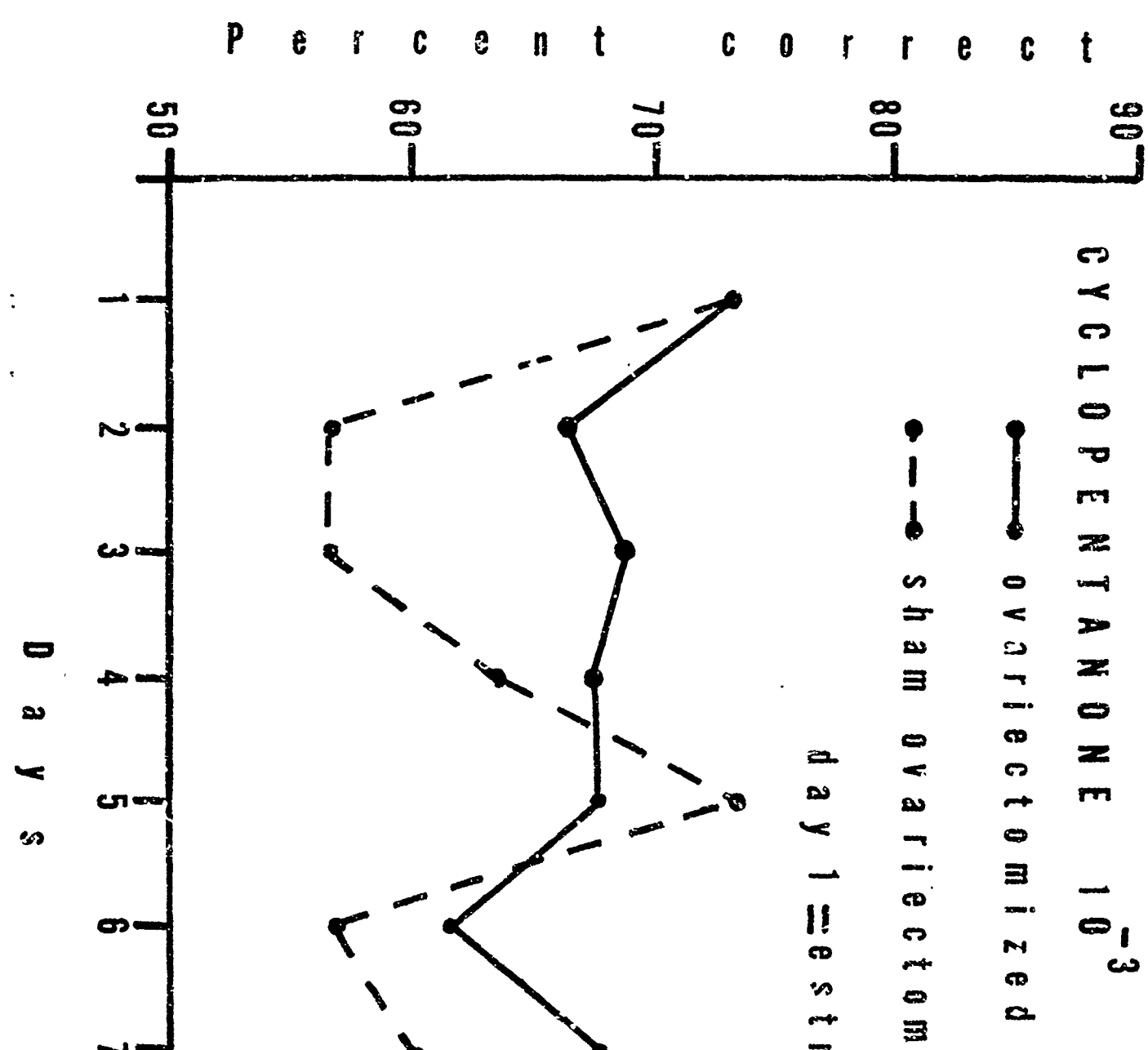


Fig. 15 Influence of ovariectomy on the responsiveness of rats to cyclopentanone (10^{-3})

variations in the daily performance of the control group are statistically significant ($p<.05$) while those of the ovariectomized are not. Furthermore, a comparison of pre-ovariectomy trials with vaginal estrus or the projected estrus day in the case of the latter trials ($p<.001$) while performance differences are not significant for the other days. The performance of the ovariectomized females was lower than that of the normal males tested previously in experiments mentioned above ($p<.001$). The performance of the ovariectomized females is also significantly greater than that of the PSP group (p .001). That the flattening of the estrus cycle in the experimental group is not more pronounced seems to be due as much to the depression of the cycle in the sham control group. Surgical trauma in one of the three sham rats may have been responsible for the observed depression of the curve for this normal group. Nevertheless, the overall effect is one that suggests a confirmation of the initial hypothesis.

Influence of testosterone on performance of ovariectomized female rats

Earlier experiments reported above show that the olfactory acuity of male rats does not fluctuate significantly.

To determine the possible influence of androgens on performance in an odor detection task, testosterone was administered to ovariectomized females.

Ovariectomized rats used in previous experiments were divided

into two groups. Five rats in the experimental group were each given 1.0 mg of testosterone propionate/ 0.1 cc sesame oil (intramuscular) per day approximately 20 hours before each experimental session for five consecutive days. After the fifth day, the dosage level was increased to 5.0 mg TP/ 0.1 cc sc per day for a further five day period. The second group of 3 rats served as a control group. They received daily injections of 0.1 cc sesame oil, the vehicle for the TP in the experimental group, during the 10 consecutive days of the experiment.

It is immediately clear from Figure 16 that the administration of testosterone propionate markedly enhances the performance of female rats on an odor detection task. The performance of the experimental group was significantly higher than that of the control group ($p<.0005$). Furthermore, the response appears to be dose dependent since the enhancement is more striking in the case of the rats receiving 5.0 mg/ day dose levels than those receiving 1.0 mg/ day. Indeed, all rats in the second experimental series attained a level of 100% correct on the final day of the experiment.

It is important to note that the levels of TP in the second series of experimental sessions were in the supranormal physiological range even for normal male rats. The performance level of the 5.0 mg TP group was significantly higher than that of normal males tested previously ($p<.0005$). The 1.0 mg TP group had performance levels not significantly different from those of normal males in earlier experiments ($p<.001$).

Fig. 16 Influence of testosterone on the responsiveness of ovariectomized rats to cyclopentanone (10^{-3})

A further analysis of previous results also shows that normal males perform at significantly higher levels than ovariectomized females ($p<.0005$). This information reinforces the conclusion from the TP injection experiments that androgens seem to facilitate performance in odor detection experiments.

DISCUSSION

The purpose of this study was to clarify the long-standing contention that mammalian hormones can influence the level of olfactory acuity. The results of experiments outlined here suggest the following general conclusions:

(1) Male rats do not show significant differences in olfactory acuity in time.

(2) The performance of female rats in an odor detection task shows significant fluctuations in time which correlate with normal fluxes in endocrine function during the estrus cycle.

(3) The observed response variations in the ♀ do not appear to be specific for any one odor stimulus.

(4) The response changes seem to be induced by ovarian hormones because performance variations in ovariectomized females are not significant.

(5) Olfactory acuity of pseudopregnant females is significantly less than that of ovariectomized females.

(6) During the estrus cycle, olfactory acuity rises in the preovulatory phase, peaks in the time around ovulation and falls again in the postovulatory phase.

(7) These observed fluctuations in olfactory acuity correlate well with previously measured changes in the plasma concentrations of

estrogen and progesterone, and such data suggest a synergistic role for these ovarian hormones in effecting the observed fluctuations in olfactory acuity.

(8) Androgen-treated ovariectomized rats show a significant increase in olfactory acuity which is dose-dependent.

These conclusions are supported, in part, by the work of previous investigators. Le Magnen (1952a) and Köster (1965) have shown that olfactory sensitivity increases significantly in the time around ovulation in humans. Our data, however, do not support Le Magnen's claim that the phenomenon is specific for only one odor but does agree with the findings of Köster (1965), Schneider and Wolff (1955), and Carr and Caul (1962) which show that the effect is not stimulus-bound. Furthermore, contrary to later findings of Le Magnen (1952b), this study shows that such olfactory phenomena also occur in the female rat.

Previous studies (Le Magnen, 1952b) with the female white rat have shown that ovariectomized females have lower olfactory thresholds for a number of odors than normal females. Using better stimulus controls, our data indicate that the opposite relationship is true and is supported by the findings of Cluzel (1964) with castrated women and Schneider et al. (1958) with hypogonadal women.

Le Magnen also found significant changes in olfactory sensitivity in ovariectomized rats that had been treated with estradiol or testosterone (1952b). The results with estrogen treatment at dose levels much higher than in our study are inconsistent. His findings

that androgens significantly increase olfactory acuity in the castrated female are in substantial agreement. In contrast to these results, Schneider et al. (1958) reported that androgen treatment in the normal physiological range depresses olfactory acuity in hypogonadal women, but this study was based on findings from only one subject. With systemic injections of 600-1000 ug TP in the male rat, Pfaff and Pfaffmann (1969) found that the magnitude of single unit responses in the olfactory bulb was enhanced in the excitatory direction in most experiments. Simultaneous recordings of spontaneous wave activity always showed larger amplitude olfactory bulb waves. They noted that these changes after testosterone tended to be greater for female urine odors than for non-urine odors. These electrophysiological results serve to reinforce our finding that systemic TP injections in the ovariectomized female can increase the level of behavioral response to an odor stimulus.

Our experiments with normal female rats demonstrate that odor detection responses rise significantly in the preovulatory phase and decline significantly in the postovulatory phase of the estrus cycle. Furthermore, the peak of the response change was found to occur between the time of late proestrus and early estrus or around the time of ovulation. These response fluctuations were also shown to correlate well with measured changes in the titers of estrogen and progesterone during the estrus cycle. In addition, response fluctuations were observed to cease in ovariectomized and

pseudopregnant rats. The electrophysiological studies of Sawyer (1971) tend to support these findings. With the intraventricular administration of norepinephrine which is known to induce ovulation, Sawyer found a prolonged increase in the amplitude and frequency of EEG recordings in the olfactory bulb of rabbits. The bulbar activity changes did not occur with pseudopr[illegible]t animals but were observed to reappear with the return of normal estrus. These combined behavioral and electrophysiological measures of the function of the olfactory system in divergent hormonal states strongly implicates that the observed effects are attributable to gonadal hormones.

Indications of the specific roles for estrogen, progesterone and androgen can be found in our data. It has been established that only a combination of estrogen and progesterone can induce and maintain the decidual growth in the uterus which is characteristic of pseudopregnancy (Yochim and DeFeo, 1962), but the estrogens also help to maintain the condition by increasing the synthesis and release of prolactin from the pituitary (Everett, 1964; Raimerez and McCann, 1964; McDonald et al., 1969). In such an artificially induced pregnancy when the titer of estrogen is generally at about the level in early diestrus (McDonald, 1969; Yoshinaga, 1969), and the titer of progesterone ebbs and flows (Hashimoto, 1968), no significant variation in the level of response to odor was observed. Furthermore, the response level was significantly less than that of ovariectomized females ($p<.0005$) and even normal females in the postovulatory phases of metaestrus and diestrus. These results

indicate that progesterone may induce a decrease in olfactory acuity. Progesterone is known to exert depressant effects on various central nervous processes (Heuser, 1967; Kawakami and Sawyer, 1959; Banerjee, 1971). Reports from other investigators also indicate that estrogen alone can significantly increase olfactory acuity in hypogonadal women (Schneider, 1958), and, alternatively, the observed decrease in odor response could be explained to result from the relative decrease in the plasma concentrations of estrogen. Nevertheless, these abnormal hormonal states may not accurately reflect those changes occurring in the normal cycling animal. The results of slope variance analyses of the plasma concentration curves of both estrogen and progesterone with odor response variations during the estrus cycle reveal that the difference between these respectively paired slopes is not significant. It is, therefore, possible that estrogen plus progesterone may act synergisticly in the normal female to induce changes in the level of odor acuity. Precedents for this latter interpretation can be found in the results of hormonal influence upon other sensory modalities (Vogel, 1971) and behavioral systems (Meyerson, 1970; Kawakami and Sawyer; Rodgers, 1970; Barfield and Lisk, 1970). Further experiments to determine the effects of A and B derivatives of estrogen and progesterone, alone and in combination, in ovariectomized animals are necessary to clarify these relationships. However, with regard to the androgens, it

seems clear from our data that they effect increases in the level of olfactory acuity which are dose-dependent. An ovariectomized female receiving daily injections of TP at a concentration of 1.0 mg shows an odor detection response which does not vary significantly in time and which is, indeed, not significantly different from that of normal males. This androgen effect, therefore, would seem to explain why male rats do not differ significantly in their performance from day to day in contrast to the high significant variations shown by the females during the estrus cycle ($p<.01$).

One question not answered by this study concerns the level at which the observed effects occur. Correlates of the rat sexual cycle include variations in such diverse phenomena as mucosal water and sodium transfer; rate of cell proliferation in salivary glands; unit activity in the hypothalamus; and suppression of food intake and bar-press duration in a light contingent situation. Consequently it is possible to envisage multiple levels at which performance in an odor-detection task might be modified. Available evidence does not allow us to favor any one site over the other, and further studies will be needed to narrow down the range. There is no good evidence to support the implied assumption of earlier studies that such effects occur at the receptor level. The only possible exception to this in the case of studies reporting that in women, fluctuations in

sensitivity to Exaltolide were of greater amplitude than those found for other odors. The present studies, however, suggest that this conclusion is not valid for the rat.

BIBLIOGRAPHY

Banerjee, V. (1971). Neuroendocrinology, 7, 27.

Barfield, M.A. and Lisk, R.D. (1970). Endocrinology, 87, 1096.

Carr, W.J. and Caul, W.F. (1962). Animal Behav. 10, 20.

Cluzel, J.C.J. (1964). These Marseille (cited by Guerrier et al, 1969).

Everett, J.W. (1964). Physiol. Rev. 44, 373.

Gilmore, D.P. and McDonald, P.G. (1969). Endocrinol. 85, 946.

Guerrier, Y., Azemar, R., and Morineau, Ch. (1969). Olfactalogia 1, (2), 1-7.

Hashimoto, I., Hendricks, D.M., Anderson, L.L., and Melampy, R.M. (1968). Endocrinology, 83, 333.

Heuser, G. (1967). Anesthesiology, 28, 173.

Kawakami, M. and Sawyer, C.H. (1959). Endocrinology, 65, 652.

Kloek, J. (1961). Psychiat. Neurol. Neurochir. 64, 309.

Köster, E.P. (1965). Int. Rhinol. 3, 57.

Köster, E.P. (1968). Olfactalogia, 1, (1), 43-51.

Le Magnen, J. (1952a). Arch. Sci. Physiol., 6, 125.

Le Magnen, J. (1952b). Arch. Sci. Physiol., 6. 295.

Meyerson, B.J. (1968). Nature 217, 683.

Moulton, D.G., Celebi, G., and Fink, R.P. (1970). In Wolstenholme, G.E.W. and Knight, J. (eds.), Ciba Foundation Symposium on Taste and Smell in Vertebrates, 227-250.

Moulton, D.G. and Pietras, R. In Le Magnen, J. (ed.). Sens chimiques et alimentation L'Association Naturalia et Biologia (in press).

Pietras, R. and Moulton, D.G. (1969). Paper given at East-Central Conference in Comparative Endocrinology. Villanova University, Pennsylvania, U.S.A.

Pfaff, D.W. and Pfaffmann, C. (1969). Brain Research, 15, 137.

Ramirez, V.D. and McCann, S.M. (1964). Endocrinology, 75, 206.

Rothchild, I. and Schwartz, N.B. (1965). Acta Endocr. (Copenhagen), 49, 120.

Schneider, R.A. and Wolf, S. (1955). J. Appl. Physiol., 8, 337-342.

Schneider, R.A., Costiloe, J.P., Howard, P., and Wolf, S. (1958). J. Clin. Endocrinol. and Metab., 18, 379-390.

Siegel, S. (1956). Nonparametric Statistics for the Behavioral Sciences. New York, McGraw-Hill.

Vogel, W., Braverman, D.M., and Klaiber, E.L. (1971). Science, 172, 388.

Yochim, J.M. and DeFeo, V.J. (1962). Endocrinol., 71, 134.

Zarrow, M.X., Jochim, J.M., and McCarthy, J.L. (1964). Experimental Endocrinology. New York, Academic Press.